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Acta Pædiat Scand 63 1–8 1974

PYROGLUTAMIC ACIDURIA

Studies in an Infant with Chronic Metabolic Acidosis

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In 1970, Jellum et al. (8) reported a 19-year old mentally retarded male suffering from spastic tetraparesis and ataxia who after abdominal surgery developed a life-threatening metabolic acidosis. The patient was found to excrete large amounts of pyroglutamic acid in the urine. The possible metabolic block was suggested to be located in the breakdown of pyroglutamate and this hypothesis was tested by studies on tissue cultured fibroblasts (14) Cells from the patient, however were found to contain normal levels of 5-oxo-prolinase.

We have recently diagnosed another case of pyroglutamic aciduria in a 1 year-old girl with metabolic acidosis. To further elucidate the mechanism of this inborn error of metabolism we have studied the effect of varying protein intake on the excretion of pyroglutamate, the turnover of pyroglutamate as well as the presence of 5-oxo-prolinase in isolated leucocytes. We have tested the hypothesis that pyroglutamic aciduria is due to an enzymatic block in the γ-glutamyl cycle, which was postulated by Orlowski & Meister (11) as a model for the active transport of α-amino acids across cell membranes.

CASE HISTORY

The patient is a girl who was referred to our hospital at 11 and 14 months of age because of chronic metabolic acidosis. She is the only child of healthy non-related parents.

The patient was born at term after an uncomplicated pregnancy The birth weight was 2 700 g. On the third day of life the baby developed a severe metabolic acidosis, which was corrected by intravenous administration of sodium bicarbonate. Attempts to discontinue the bicarbonate therapy resulted in a return of the acidosis. The girl has required 30 mmoles of sodium bicarbonate daily ever since.

The girl was fed standard baby formula for 5 months and after that she received ordinary baby food. Her self-adjusted diet has then contained 4 g of protein per kg body weight and day and the history did not reveal intolerance to protein-rich meals.

Physical examination at 11 and 14 months of age revealed a healthy looking girl with normal weight and height for her age. There were no signs of neurological or other abnormalities. The psychomotoric development was normal (DQ 115 according to Buhler-Hetzer).

Routine examinations of blood and urine gave normal results, except for the presence of a compensated metabolic acidosis. On a daily intake of 30 mmoles of sodium bicarbonate, repeated investigations of the patient's acid-base balance in the blood gave the following results: pH 7.30–7.45 pCO_2 31–43 mmHg; standard bicarbonate 17 23 mM base excess −2 to −7.5 mEq/litre. The pH of the urine varied between 4.7 and 5.9

Studies of her renal function showed normal glomerular filtration rate (110 ml/min and 1.73 m^2 body surface) and normal tubular reabsorption of bicarbonate.

The levels of α-amino acids in plasma and urine were found to be normal, and the urine contained insignificant amounts of protein. The serum level of γ-glutamyl transpeptidase was within the normal range.

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The presence of pyroglutamate in the urine was established as described in the Results section

MATERIAL AND METHODS

Uniformly ^{14}C-labelled L pyroglutamate and L-glutamate were from New England Nuclear Chemicals (Dreieichenhain West Germany).

Ether extraction of organic acids from acidified urine conversion to methyl derivatives and separation by gas chromatography was performed as described by Hagenfeldt (6) Gas chromatography of the tri methylsilyl (TMS) derivative was used for the quantitative measurement of pyroglutamic acid. Plasma was then deproteinized either by acidification or by centrifugation through a Diaflo® ultrafilter Norvaline was added as an internal standard. Urine or deproteinized plasma was evaporated to dryness and silylated by bis(trimethylsilyl)trifluoroacetamide at 125 for 15 min. Under these conditions pyroglutamic acid was converted to its mono-TMS derivative and glutamic acid to its tri TMS derivative Gas chromatography was performed according to Bergström & Gürtler (1)

Mass spectra were obtained with a LKB 9000 GLC MS Instrument (LKB Products, Stockholm, Sweden)

An additional method for determination of L pyroglutamate was based on acid hydrolysis to L glutamate Urine or deproteinized plasma samples were hydrolysed in 4 M HCl at 100° for 3 hours. This resulted in a complete conversion of pyroglutamate to glutamate which was assayed according to Williamson & Corkey (17) Glutamine and glutamate present in the original sample were determined separately in the automatic amino acid analyzer The amount of L pyroglutamate in the original sample was calculated by subtracting the amounts of glutamate and glutamine in the sample prior to hydrolysis. In urine the levels of glutamate and glutamine were insignificant compared with that of pyroglutamate, but in plasma they amounted to 10–20% of the pyroglutamate.

The turnover of pyroglutamic acid was studied in the patient by an intravenous injection of 5 μCi of ^{14}C pyroglutamate (spec act 200 mCi mmole) Blood samples were withdrawn at 60 min intervals. The specific activity (dpm/μmole) of pyroglutamic acid in plasma was determined after isolation of the compound as glutamate according to the following procedure, protein was precipitated by boiling and removed by centrifugation. The supernatant solution was passed through a column of Dowex 50-H^{+} and the material eluted with water was collected This fraction containing pyroglutamate was lyophilized and subsequently hydrolysed in 4 M HCl at 100° for 3 hours. The glutamate formed was rechromatographed on a column of Dowex 50-H and eluted with 3 M NH_4OH as described by van der Werf et al (16) The specific activity of the isolated glutamate was determined and taken as a measure of the specific activity of pyroglutamate present in the original sample.

Leucocytes were isolated and incubated as described elsewhere (7) using ^{14}C-pyroglutamate or ^{14}C glutamate as substrate Cell-free extracts of leucocytes were prepared by suspending approximately 3×10^7 cells in 50 mM Tris-acetate buffer pH 7.5 containing 5 mM dithiothreitol and 5 mM L pyroglutamate. The cells were disrupted by sonication in a Bronwill Biosonic Model 2 at 20% output for 30 sec The sonicate was centrifuged at 30 000 g for 30 min, and the supernatant solution passed through a Sephadex G 25 column pre-equilibrated with 50 mM Tris acetate, pH 7.5 containing 5 mM dithiothreitol The protein material eluted with the void volume was used for studies of 5-oxo-prolinase The conditions for the incubation were those described by van der Werf et al. (16) except for the fact that the specific activity of ^{14}C L pyroglutamate was increased to 1.5×10^6 cpm/μmole

Protein was measured as described by Lowry et al. (10)

RESULTS

Identification of L pyroglutamic acid excreted in the urine

A sample of the patient's urine was acidifed and extracted with ether After methylation of this ether fraction gas chromatographic analysis revealed the presence of large amounts of a compound which was not detected in urine of control individuals. The mass spectrum of this material was identical to that of authentic L pyroglutamic acid methyl ester

Quantitatively the procedure involving gas chromatography of methylated ether extracts, was not satisfactory mainly due to difficulties to obtain reproducible extraction of pyroglutamic acid into the ether phase The quantitative results with the method using TMS-derivatives were reproducible and agreed within ±5% with the results of the enzymatic determination of L glutamate after acid hydrolysis.

Under standard conditions the average daily excretion of pyroglutamate in the urine was found to be 51 mmoles (range 48 to 54 mmoles)

In order to determine the configuration of the excreted acid urine samples were subjected to acid hydrolysis to convert the pyroglutamic acid to the corresponding glutamic acid. The amount of L glutamic acid present after hydrolysis was analysed using glutamic acid dehydrogenase which is strictly specific

for the L-configuration (13). After hydrolysis, pyroglutamate was quantitatively recovered as L-glutamate, indicating that pyroglutamate excreted was exclusively of the L-configuration.

Urine samples were also subjected to high voltage paper electrophoresis before and after hydrolysis with 4 M HCl for 3 hours. Ninhydrin spray was used to detect amino acids. Before hydrolysis only normal amounts of free α-amino acids were found, and there was no indication of the presence of oligopeptides. Hydrolysis resulted in a substantial increase of glutamate whereas the amounts of other amino acids remained constant.

We also considered the possibility that pyroglutamate was formed as an artefact from some precursor in the patient's urine. This was considered unlikely for several reasons. The urine samples were immediately frozen after collection. The amounts of pyroglutamate in the individual samples remained constant even upon storage either at +4 or frozen at −20 for several months. Similar handling and analysis of urine from a large number of other individuals, including the patient's mother has not shown the presence of pyroglutamate.

Effect of varying protein intake on urinary excretion of pyroglutamic acid

In order to study the effect of changes in the protein intake on the excretion of pyroglutamate, the patient was given different diets containing 2, 4 and 6 g of protein/kg body weight and day respectively The effect on the excretion of nitrogen metabolites in the urine is shown in Table 1

The production of urea varied in relation to the protein load. The excretion of pyroglutamate, on the other hand, seemed to decrease with increasing protein intake the significance of this observation remains to be established. The amounts of ammonia excreted were rather low with respect to the acidosis. The sum of urea, pyroglutamate and ammonia corresponded to more than 90% of the total nitrogen excreted in the urine.

Table 1 *Nitrogen balance studies on diets containing different amounts of protein expressed in mg N/24 hours*

	Period I	Period II	Period III
Dietary intake	2 850	5 900	8 850
Urinary excretion			
Total nitrogen (Kjeldahl)	2 540	4 720	6 900
Urea	1 220	3 290	5 370
L-pyroglutamate	840	725	610
Ammonia	280	375	290
Creatinine	64	60	67
Creatine	15	35	43
Uric acid	7 7	23	7 7
Faecal excretion	—	480	—

Period I. diet containing 2 g of protein/kg B.W and day
Period II diet containing 4 g of protein/kg B.W and day
Period III diet containing 6 g of protein/kg B.W and day
The patient obtained the respective diet during 2 days before its effect on the nitrogen balance was studied.

On her normal diet of 4 g protein per kg body weight and day pyroglutamate accounted for approximately 15% of the total nitrogen excretion. The net intestinal absorption of dietary amino acids seemed intact since less than 10% of the ingested protein nitrogen appeared in the faeces (Table 1).

Presence of pyroglutamate in plasma and its renal clearance

The fasting levels of pyroglutamate in plasma were constantly found to be 4.5–5 0 mM which corresponded well to the average base excess of −5 mEq/L

The renal clearance of pyroglutamate was 7.5 ml per minute compared with a glomerular filtration rate of 24 6 ml per minute. Thus, substantial reabsorption of pyroglutamate seemed to occur in the kidneys.

Effect of peroral protein load on the plasma level of pyroglutamate

In order to study the effect of the level of α-amino acids in plasma on the plasma concentration of pyroglutamate the patient was fasted overnight and then given a single meal

Table 2. *Effect of per oral protein load on plasma levels of pyroglutamate and α-amino acids*

Hours post prandially	α-amino acids (mM)	Pyroglutamate (mM)
0	5.20	4.55
1	—	4 76
2	10 1	4.56
3	—	4 70

containing 2 g of protein/kg body weight. This resulted in a doubling of the free α amino acid levels in plasma 2 hours after the meal (Table 2) The most pronounced increments were found for valine proline leucine and isoleucine. During 3 hours postprandially the plasma levels of pyroglutamate remained constant indicating that there was no immediate effect of free α amino acids on the concentration of pyroglutamate in plasma

Clinically the patient was unaffected by the protein rich meal and her acidosis remained well compensated

Turnover of pyroglutamate

The turnover of pyroglutamate was studied by giving an intravenous injection of ^{14}C-pyroglutamate Steady state conditions were established by fasting the patient overnight The specific activity of plasma pyroglutamate at different times after the injection is shown in Fig. 1 The specific activity decreased according to first-order kinetics with a half life of 2 4 hours, corresponding to a turnover time

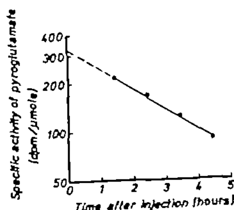


Fig 1 Specific activity of plasma pyroglutamate after injection of ^{14}C-pyroglutamate

Table 3 *Activity of 5-oxo-prolinase in cell free extracts of leukocytes*

Source of enzyme	5-oxo-prolinase (mU/mg of protein)
Patient	0.69
Normal controls	0.27 0.48

of 3.5 hours. Based on the total amount of *radioactivity administered and the specific ac*tivity of pyroglutamate extrapolated to zero time (Fig 1) we estimated the total pool of pyroglutamate to be 31 mmoles. This would give a daily synthesis of approximately 210 mmoles of pyroglutamate The daily excretion in the urine on the other hand amounted to only 50 mmoles, indicating endogenous break *down of 75% of the pyroglutamate synthe*sized.

Breakdown of pyroglutamate in leucocytes

Further studies on the patient's ability to metabolize pyroglutamate were carried out with isolated leucocytes. The conversion of ^{14}C pyroglutamate to $^{14}C-CO_2$ was taken as an index of pyroglutamate degradation. Leucocytes from the patient, her mother and normal controls were all found capable of metabolizing pyroglutamate, but no *significant* difference between the cells was observed. Variations in the size of the intracellular pools of pyroglutamate might however mask true differences in enzymatic ability between the patient and the control individuals. The results could therefore with confidence be regarded only as a qualitative measure of the relevant metabolic pathway

The initial step in pyroglutamate catabolism is catalysed by 5-oxo-prolinase (L pyroglutamate + ATP→L-glutamate + ADP + P_i) Cell-free extracts of leucocytes were analysed for their content of this enzyme. By gel filtration of the extracts prior to analysis of enzyme activity differences due to variations in internal pyroglutamate pools were eliminated

The results are shown in Table 3 No deficiency of 5-oxoprolinase could be demonstrated

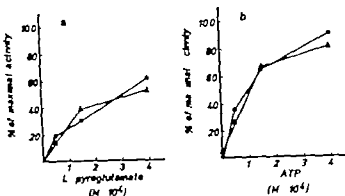


Fig 2 Substrate concentration curves for 5-oxo-prolinase. (*a*) L-pyroglutamate. (*b*) ATP ●—● enzyme from leucocytes of the patient. ▲—▲ enzyme from leucocytes of control individuals.

in leucocytes from the patient· if anything, her levels were higher than those of the two control individuals. Furthermore, the affinity of the patient's enzyme for its substrates, L pyroglutamate and ATP did not seem to differ from that of enzyme from controls (Fig. 2).

DISCUSSION

Pyroglutamic aciduria was described by Jellum et al. in 1970 (8) Their patient was a 19-year-old mentally retarded male with spastic tetraplegia and cerebellar ataxia. Following an operation for hiatus hernia a chronic metabolic acidosis was diagnosed. Large amounts of pyroglutamate were excreted in the urine.

Our patient with pyroglutamic acaduria presented symptoms of metabolic acidosis already on the third day after birth. Ever since then she has been on continuous treatment with bicarbonate to correct the acidosis. At an age of 14 months she was normally developed and showed no signs of neurological disease. The acidosis seemed to be related to the excretion of L-pyroglutamate in the urine.

The daily urinary excretion in our patient corresponded to 210 mmoles/1 73 m^2 body surface which is in good agreement with the excretion of 240 mmoles observed in the previously reported adult male (8).

Several pieces of evidence indicate that L pyroglutamate is a normal metabolite in mammals. Many tissues contain potent enzyme systems for its synthesis and breakdown (12). L-pyroglutamic acid occurs as the *N*-terminal residue in several polypeptides including fibrinogen (2) immunoglobulins (3) and collagen (9) Urinary excretion of pyroglutamate in humans was first observed by Tham et al. (15) in patients with burns and allergic diseases. The amounts were however only a few mg per day

In their original report Jellum et al. (8) suggested that the high urinary excretion of pyroglutamate might be due to an enzymatic defect in the metabolic pathway responsible for the conversion of amino acid nitrogen to urea. This hypothesis was tested on our patient by studies of her urea metabolism. However since her plasma levels of urea, ammonia and α-amino acids were normal and the urinary excretion of urea varied with the protein intake we consider a primary defect in the urea synthesis as highly unlikely These findings confirm recently published results on the adult patient with pyroglutamic aciduria (4).

The turnover of pyroglutamate in our patient indicated that she was able to metabolize approximately 75% of the amount synthesized. For comparison, the adult patient excreted about 30% of an intravenous dose of ^{14}C-pyroglutamate unchanged in the urine in 24 hours (4). With the lack of data from normal individuals it is impossible to distinguish between overproduction and defective catabolism of pyroglutamate but the working hypothesis of a partial defect in the degradation of pyroglutamate has been tested.

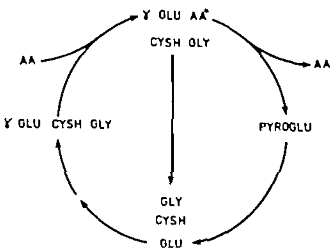


Fig 3 The γ-glutamyl cycle. For details see text.

On the basis of the findings of high activities of the enzymes which are involved in the degradation of pyroglutamate in renal tissue Orlowski & Meister (11) have proposed a model called the γ-glutamyl cycle (Fig 3) which offers a metabolic role for pyroglutamate in the transport of α-amino acids across cell membranes. In the proximal renal tubules α-amino acids are reabsorbed by an active process involving the transfer of the γ-glutamyl residue of glutathione (γ-GLU-CYSH-GLY) to a variety of amino acids (AA*) by the action of the enzyme γ-glutamyl transpeptidase which has been shown to be localized to the brush border (5) The dipeptides formed (γGLU AA*) are hydrolysed by the cytoplasmatic enzyme γ-glutamyl cyclotransferase into free amino acids and pyroglutamate and the latter compound is subsequently converted to glutamate by 5-oxo-prolinase an enzyme which has recently been isolated from rat kidney (16)

It is tempting to speculate that pyroglutamic aciduria is caused by a defect in the conversion of pyroglutamate to glutamate in the kidneys, i e. that there is a block in the γ-glutamyl cycle. The fact that the tubular reabsorption of α-amino acids remains intact as revealed from the absence of abnormal aminoaciduria could be explained on the basis that the loss of pyroglutamate is compensated for by a supply of glutamate

We have not, however obtained any experimental support for the production of pyroglutamate in the kidneys. The clearance of pyroglutamate was only 30% of the glomerular filtration rate. When the tubular load of α-amino acids was increased by doubling their plasma concentration the level of pyroglutamate in plasma remained constant (Table 7) and when the protein intake was increased there was no increase of the amount excreted in the urine. On the other hand Eldjarn et al (4) when trying to find evidence for a block in the γ-glutamyl cycle have demonstrated a doubling of the urinary excretion of pyroglutamate in their adult patient after a 5-fold increase of the levels of amino acids in plasma obtained by massive intravenous infusion. This discrepancy between the 2 patients might be explained by the difference in plasma amino acid levels in the two experiments. The results of Eldjarn et al (4) do not necessarily imply a block in the γ-glutamyl cycle since the increased excretion of pyroglutamate might be due to a decreased tubular reabsorption of pyroglutamate because of competition with α-amino acids.

In studies on tissue cultured fibroblasts from their patient with pyroglutamic aciduria, Strömme & Eldjarn (14) have found that the conversion of ^{14}C-pyroglutamate to ^{14}C-CO_2 occurs at a normal rate and that there was no 5-oxo-prolinase deficiency In confirmation with these findings we have demonstrated that the 5-oxo-prolinase activity in cell-free extracts of leucocytes from our patient was not decreased. Furthermore, the enzyme from the patient and controls did not differ with respect to their affinity for L pyroglutamate and ATP Thus, both patients with pyroglutamic aciduria studied so far appear to have at least one intact structural gene coding for 5-oxo-prolinase. The possible existence of tissue specific isozymes of 5-oxo-prolinase has not yet been elucidated. Alternative mechanisms for the formation of pyroglutamic acid might also be considered, for instance via an altered glutamine synthetase.

The reason for the metabolic acidosis in our

patient is not quite clear One explanation might be that there is a shunting of α-ketoglutarate from the citric acid cycle since this metabolite is a likely precursor for glutamate and hence pyroglutamate. A defective citric acid cycle would ultimately result in ketoacidosis Another explanation for the acidosis might be that pyroglutamate acts as an inhibitor on one or more metabolic processes. Therapeutically our patient has been continuously substituted with bicarbonate and maintained on a high protein intake. We have been unable to affect the plasma levels or urinary excretion of pyroglutamate.

So far it has not been possible to demonstrate any metabolic block either in the adult patient reported by the Norwegian workers or in the child studied by us. Although the primary biochemical defect remains obscure pyroglutamic aciduria may fulfil the criteria of an inborn error of metabolism. However in both cases is the family history non-confirmatory of a genetically determined disorder

SUMMARY

A one year-old girl with chronic metabolic acidosis was found to have normal renal acidification. In the urine, however the excreted a daily amount of 50 mmoles of L-pyroglutamate (5-oxo-L-proline). This excretion did not increase with increasing protein intake. Studies of the turnover of L-pyroglutamate under steady state conditions revealed that 75% of the pyroglutamate synthesized was metabolized by the patient. The level of pyroglutamate degrading enzyme (5-oxo-prolinase) in the patient's leucocytes was not decreased, and the enzyme appeared to have normal affinity for its substrates, i.e. pyroglutamate and ATP

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MUCOLIPIDOSIS II (I-CELL DISEASE)

A Clinical and Biochemical Study

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Mucolipidosis II (I-cell disease) is a clinical condition which is characterized by psychomotor retardation, shortness of stature and a Hurler-like appearance there is, however a normal excretion of acid mucopolysaccharides (AMPS) in the urine. The diagnosis is based on the presence of cytoplasmic granules in cultured skin fibroblasts observed under phase microscopy and on a multiple deficiency of lysosomal acid hydrolases of the cultured cells (11 12). It is supposed that the disorder is inherited by means of an autosomal recessive gene (11). Until now about 14 patients have been described which show the clinical and biochemical characteristics of this disorder (19).

The reduction of multiple lysosomal enzyme activities in the fibroblasts has been shown to be accompanied by the increased activity of the same enzymes in the conditioned culture medium (24). In order to verify this observation in the patient we have analysed the extracellular fluids of the patient who was available for a clinical study Since the findings shed a new light on the pathogenetic mechanism involved in I-cell disease, the clinical history and the follow up of the patient will be given in detail in this paper

The possibility of a correction of the elevated enzyme levels was tested in the plasma with several drugs which are known to stabilize lysosomal membranes *in vivo* and *in vitro* (17 23). Since a multiple lysosomal enzyme deficiency in cultured fibroblasts prevents the normal degradation of various compounds in the lysosomes (25), we analysed the clinical and histological material for evidence of a depletion of lysosomal enzymes in the cells of the patient.

METHODS

Urinary mucopolysaccharides: Mucopolysaccharides were extracted by a batch procedure from 10 ml urine from a 24-hour collection. 4 g of dry activated ecteola (chloride form) powder was added to 10 ml of urine. The ecteola was washed three times with 20 ml of 0.9% saline and the MPS extracted twice with 20 ml of 3 M NaCl. 20 ml of the eluate was dialysed exhaustively against distilled water and total hexuronic acid was determined by the modified Carbazol reaction according to Bitter & Muir (4).

Urinary cerebroside sulfate (Sulfatide): Urinary sulfatide was estimated by the method described by Herschkowitz et al. (7).

Isolation of peripheral leucocytes: The isolation of polymorphic leucocytes from the peripheral blood was performed according to Percy et al. (15). The washed cells were resuspended in 2 ml of distilled water and sonicated twice for 30 min in a Branson sonicator (Branson Sonic Power Co., Danbury Conn.).

Determination of lysosomal hydrolases. Urine, plasma and CSF were dialysed over night against distilled water prior to enzyme determinations. Arylsulfatase A was determined according to Austin et al. (2). The enzyme activity was expressed as 1 U = 1 µmole substrate cleaved per min at 37°C. *β*-galactosidase, *α*-fucosidase and *N*-acetyl-*β*-galactosaminidase were determined according to Van Hoof &

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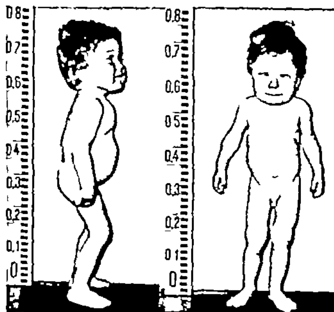


Fig 1 G G at the age of 4 years. Note the joint contractures.

Hers (20) All activities were expressed per litre extracellular fluids or corrected for cellular protein. Cell protein was measured by the method of Lowry et al. (8)

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RESULTS

Case history and clinical examinations

G G the first child of unrelated parents was born after an uneventful pregnancy The birth weight was 3 000 g length 49 cm The infant was unusually placid and held the knees constantly flexed. At 8 months he was operated on for the repair of bilateral inguinal hernias. A great fragility of the soft tissue was noted. The boy was first seen at 14 months of age and is at present 4 years old. At 14 months, the height (77 cm) weight (9.9 kg) and head circumference (47 cm) corresponded to the 50th percentile. The head circumference increased regularly during the following years along the 50th percentile. Weight and height growth decelerated to values below the 3rd percentile At 4 years of age height was 82 cm with a growth rate of 2 cm/year during the third and fourth year of life The skull is dyscephalic with a high forehead and a broad biparietal diameter The face is coarse with flat supraorbital ridges, a flat bridge of the nose and anteverted nostrils (Fig. 1) Prominent epicanthic folds and heavy protruding underlids give him a sleepy appearance. The ear lobes are fleshy the skin strikingly firm, especially in the face The gingivae are hypertrophic. At 14 months 8 incisors were erupted. At three years 16 teeth were present, no change has occured at age four The hands and feet are broad, the extension of the fingers is limited with a progressive tendency to claw hands. Flexion contractures of the hips, knee and elbow joints are progressive. A systolic murmur 2–3/6 is constantly audible over the apex. The liver slowly increased in size from 1 cm below the costal margin to 4 cm at the age of 4 years. There is no splenomegaly The corneae are clear the ocular fundi normal. Motor performance at 14 months corresponded to a developmental age of 7 months. The first steps were possible at 2 years. Besides retardation and strabismus

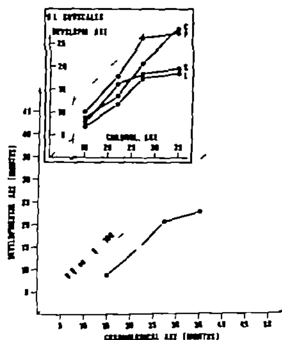


Fig 2. -- Psychometric examinations, • D Q according Brunet and Lézine· * I.Q after Kramer in the intersection. Subscales of Brunet and Lézine· *P* Postural, *C* Coordination. *L* Language *S* Social.

convergens alternans no signs of motor or sensory defects were observed at repeated neurological examinations. The results of several psychometric examinations are summarized in Fig. 2. The skeletal abnormalities are progressive (Fig. 3). They consist mainly of short and plump tubular bones, especially of the hands, a dysplasia of the pelvis with underdeveloped iliac bodies, supra-acetabular constrictions and the irregular shape of the pubis and ischium. The ribs are broad, the vertebral bodies short and rounded, with beaking in L 1 and L 2 (Fig. 3). The cranial vault is not thickened. Bone age at 4 years corresponded to chronological age.

Ancillary examinations

Nerve conduction velocities of the peroneal and ulnar nerves were measured at yearly intervals and found to be normal.

Audiometric screening reactions were normal and constant at 20 dB at frequencies between 250 and 2 000 cs/sec. EEG examinations revealed a diffuse dysrhythmia without any signs of epilepsy.

About 20% of the lymphocytes in the peripheral blood have vacuolizations of the cytoplasm with azurophilic granular inclusions. In the bone marrow 1% of the white cells were vacuolated and contained red granules in H.E. staining. These cells had the appearance of plasma cells. In a needle biopsy from the liver an increased amount of a PAS-positive material was found in the hepatocytes. This material was tentatively identified by amylase digestion as glycogen. The electronmicroscopical examination (kindly performed by Dr M. Tondeur Bruxelles) of liver tissue showed that the hepatocytes contained a small number of inclusions lined by a single membrane. Certain cells with the appearance of histiocytes, localized in the portal spaces, were enlarged and contained a considerable number of inclusions of a fine floccular material. A biopsy of the sural nerve showed a normal appearance under light microscopy. No metachromatic substances were present.

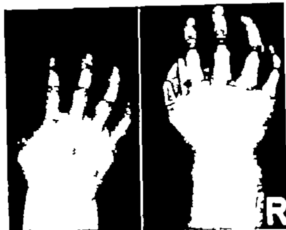




Fig. 3. X-rays of hands and spine taken at age 14 months (*left*) and 4 years (*right*). Note the progression of dysplasia of metacarpalia and phalanges and the increase in contractures of the fingers. The arrows point to the "beaking" of lumbar vertebrae L 1 and L 2.

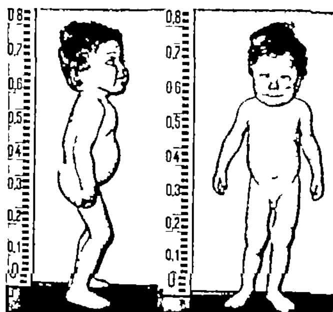


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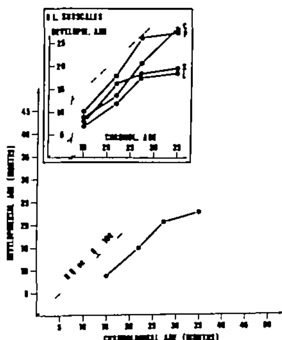


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sulfatase A and *N*-acetyl-β-galactosaminidase activities were normal compared with age matched controls.

Lysosomal enzymes in extracellular fluids

Plasma. Normal to borderline increased α-fucosidase activity was found in the plasma of patients with different types of mucopolysaccharidoses (Table 2). In the patient α fucosidase activity was 4–5 fold greater than the upper normal limits. An increase in lysosomal enzyme activity in the plasma was even better demonstrated in arylsulfatase A, where enzyme activities up to 60 times of the upper normal range were measured.

Characterisation of lysosomal enzymes in extracellular fluids

No difference between the pH optimum or the heat inactivation curves of arylsulfatase A, *N* acetyl-β-galactosaminidase and α-fucosidase in the patient's plasma and plasma from normal controls could be established (Table 3)

Effect of various drugs on activity of arylsulfatase A in extracellular fluids

Phenergan (diamethylamino-2-methyl-2-ethyl-10-phenothiazine), Vitamin E and a short-term treatment with prednisone did not affect the level of arylsulfatase A in the plasma and in the urine. The long-term treatment with high doses of prednisone caused an increase of enzyme activity in both plasma and urine (Fig. 4).

DISCUSSION

In our patient the criteria of I-cell disease was established on the basis of the clinical picture (5 9 10), the absence of a significant increase in the excretion of mucopolysaccharides in the urine and a multiple lysosomal enzyme deficiency in the cultured fibroblasts (9 11 12), which also displayed the I-cell phenomenon. The results of the electron microscopy of a needle biopsy of the liver and that of cultured fibroblasts were also consistent with this diagnosis (6).

Both parents had a 50% decreased β galactosidase activity in the skin and increased lysosomal enzyme activities in the plasma (27)· the inheritance of the disorder by our patient is therefore compatible with an autosomal recessive transmission.

The isolated increase of activities of lysosomal enzymes in the extracellular fluids of

Table 2. *Lysosomal enzyme activities in plasma*

	α-Fucosidase Units[a]	Arylsulfatase A Units[a]	*N*-Acetyl-β-galactosaminidase Units[a]
Patient G. G	33 000 ± 1 660	25 666 ± 533	27 383 ± 8 333
Mucopolysaccharidoses			
Hurler	2 365		
Hunter	931		
Sanfilippo A			
S. L.	5 216	193	3 216
A. A.	8 600		
A. M.	7 450		
Sanfilippo B			
W U	9 600	493	4 400
D A.	3 933		
Normal children (*N* = 10)	6 333 ± 2 500	306 ± 135	1 750 ± 1 250

U expressed as nmol substrate cleaved per min per litre plasma. The values represent either the results from single determinations or mean values ±2 S.D

Table 3 *Characterisation of lysosomal enzymes in the plasma*

Enzyme	Heat inactivation % residual activity after 30 min: Patient %	Control (*N* = 8) %	pH Optimum: Patient	Control (*N* = 8)
Arylsulfatase A[a]	30	30–40	5.0	5.0
N-acetyl-β-galactosaminidase[b]	32	20–30	4.4	4.4
α-fucosidase[c]	15	20–30	5.5	5.5

[a] Arylsulfatase A 30 min, 60°
[b] *N*-acetyl-β-galactosaminidase: 30 min, 55°
[c] α-Fucosidase: 30 min, 55°

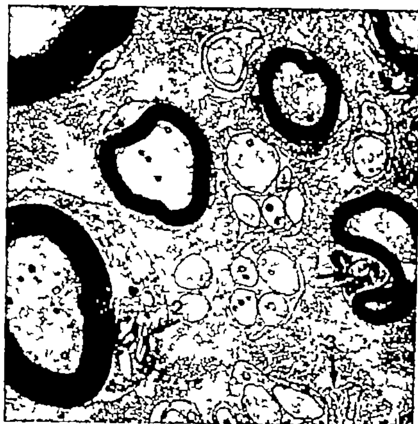


Fig 4 Suralis nerve Magnification ×9 400 Arrow 1 and 2: abnormal inclusions in lysosomes in Schwann cells of myelinated axons. The inclusions show at higher resolution circular or densely packed layers. Arrow 3 multiplication of the basal membrane in Schwann cells.

The electronmicroscopic examinations of the sural nerve revealed abnormal inclusions in the cytoplasm in about 20% of the Schwann cells (predominantly of the myelin ated axons) These inclusions were of round oval or spindle shape. At high magnification the inclusions showed either circular or densely packed layers. The inclusions were surrounded by a single membrane. They could correspond morphologically to lysosomes storing a lipid substance (Fig 4) The basal membrane of several non myelinated but also of myelinated axons was split into several layers. Mitochondria and elements of the endoplasmatic reticulum of the axons appear to be increased (Morphological examinations were kindly performed by Dr A. Bischoff Zürich/Bern)

Table 1 *β galactosidase activity in skin biopsy*

	Units
G G (Patient)	0.0
Father	10.33
Mother	13 0
Normal[b] ($N=8$)	40±25

[a] U=1 nmol substrate cleaved per min and per g cell protein.
[b] Normal values include biopsies from children and adults.

Biochemical investigations

The excretion of total acid mucopolysaccharides in the urine measured as hexuronic acid in several 24-hour urine collections was 15±4 mg/24 hour (Normal values for age-matched children ranged from 2–12 mg/24 hour) The excretion of sulfatide in the urinary sediment was 48 μg/24 hour (44 46, 55 μg). Normal values ranged from 0–22 μg/24 hour

Lysosomal enzymes in skin and in isolated leucocytes

β-galactosidase was not detectable in the skin of the patient whereas the activity of α fucosidase was 130% of normal values. In both parents the activity of β-galactosidase was reduced to 25 6% in the mother and 32.2% in the father (Table 1) In isolated leucocytes of the patient and of his parents aryl-

Lysosomal enzyme depletion could not be shown in isolated leucocytes of our patient. Decreased activities of α-galactosidase, β-glucosidase and β galactosidase but normal activity of *N*-acetyl-β-galactosaminidase have been observed in another patient (21). The absence of β-galactosidase activity in skin biopsies of our patient is not specific for this disorder but has also been observed in Hurler Hunter and Sanfilippo patients (13 26). This is also true for the reduced activity of β galactosidase in the liver of some patients with I-cell disease (11 20, 22). β-galactosidase is also found deficient in the liver of patients with mucopolysaccharidoses. The variance in the activities of lysosomal enzymes, in particular the fact that β-galactosidase is found normal in some patients and decreased in others (11) could be partially explained by the contamination of the biopsy with blood or other extracellular fluids with high lysosomal enzyme activities.

From the present data it cannot be concluded that the loss of lysosomal enzymes into the extracellular fluids causes enzyme depletion in the cells of the patient.

Even if not the absence of intracellular lysosomal enzyme activities is significant so the presence of large amount of acid hydrolases in the extracellulr fluids could be a component in the pathogenesis of I-cell disease. Extracellular action of lysosomal enzymes on the intercellular ground substance has been shown (16) to result in the alteration of the structure of function of this ground substance. This could explain at least part of the clinical picture.

SUMMARY

The diagnosis of mucolipidosis II (I-cell disease) was made in a patient with a Hurler like appearance but only borderline mucopolysaccharidurla. The cultured fibroblasts of the 2 year-old boy showed the I-cell phenomenon and multiple lysosomal enzyme deficiencies, however the activity of enzymes in the medium of the cellculture was increased. Increased lysosomal enzyme activities were also found in the extracellular fluids of the patient. Heat inactivation and pH optimum of the enzymes were normal and therefore did not suggest an abnormality in the structure of drugs known to stabilize lysosomal membranes *in vitro* had no effect on the elevated enzyme levels in the plasma of the patient, except for high doses of predníson, which resulted in a further increase in the enzyme activity On the ultrastructural level there was the storage of polymorphous material in the liver and in the peripheral nerve. Vacuolated cells were present in the peripheral leucocytes and in the bone marrow These findings indicate that the lysosmal enzyme depletion in the cells in the patient could be similar to that found in cultured cells. However the clinical picture could also be caused by the excess activity of lysosomal enzymes in the extracellular fluids which are able to degrade the matrix substance of the connective tissues and could mimic a mucopolysaccharidosis.

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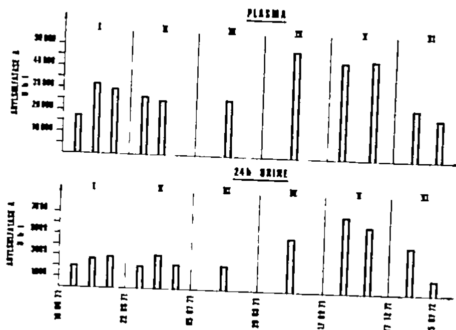


Fig 5 Effect of various drugs on arylsulfatase A activity in plasma and 24-hour urine samples. Units are given as nmoles *p*-nitrocatechol sulfate cleaved per min per liter I No Therapy II Prednisone 2 mg/kg/day III Phenergan 3 mg/kg/day IV Prednisone 3 mg/kg/day V Prednisone 1,5 mg/kg/day VI Vitamin E 10 mg/kg/day All drugs were given per os.

the patient is a new finding in this disorder it has been since confirmed by others in two I-cell patients in plasma (21) and in urine (18) In a study on the activity of lysosomal enzymes in the plasma of patients with various types of mucopolysaccharidoses, Öckermann had already observed excessive α fucosidase activity in a patient whom he considered to suffer from I-cell disease (14)

Of the drugs prednosone phenergan and vitamin E, known to act on the lysosomal membranes *in vivo* and *in vitro* (17 23) only prednisone in long term application caused an increase of arylsulfatase A activity in urine and plasma. The mechanism of the increase in activity of arylsulfatase on prednisone treatment cannot presently be explained.

Relevance of increased lysosomal enzyme activities in extracellular fluids

The increase in lysosomal enzyme activities in the plasma, urine and CFS (27) of the patient can not be attributed to a change in kinetics or conformation of these enzymes as far this would affect pH optimum or heat inactivation. The normal activity of non lysosomal enzymes seems to indicate that the increase in lysosomal enzymes is not due to cell destruction and lysis with subsequent liberation of intracellular enzymes. It might therefore be concluded that the lysosomal hydrolases are lost from apparently undamaged cells in the patient as well as in the cultured fibroblasts of the patient (27)

In cultured fibroblasts from patients with I-cell disease the intracellular activity of lysosomal enzymes is diminished therefore, ^{35}S sulfatide and ^{35}S-mucopolysaccharides cannot be normally degraded (25 26) In the patient we have but little indication that the intracellular activity of lysosomal enzymes may also be reduced. Morphological changes in lymphocytes liver cells and in cells of the peripheral nerves as well as in uncultured fibroblasts of the patient could be caused by the storage of material which cannot be degraded. The clinical picture of the patient suggests a disturbance in the metabolism of acid mucopolysaccharides. However the absence of significantly increased mucopolysacchariduria speaks against this. The increased sulfatide excretion in the urine also suggests a disturbance of the sulfatide metabolism in the kidney although the sulfatide excretion is less than that found in the urine of patients with metachromatic leucodystrophy Moreover the nerve conductivity in our patient was normal. Clinically and biochemically the patient can be clearly distinguished from cases with mucosulfatidosis (1 3)

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CONVERSION OF ANGIOTENSIN I IN PULMONARY AND SYSTEMIC VASCULAR BEDS OF CHILDREN[1]

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From the Department of Pediatrics[2] and Pharmacology Faculty of Medicine University of Montreal and l'Hôpital Sainte-Justine pour les Enfants, Montreal Canada

In vivo conversion of Angiotensin I (A I) to Angiotensin II (A II) within the lungs was first demonstrated in adult dogs by the blood-bathed organ technique (13 14) and confirmed in adult humans by the systemic pressor response assay (2, 3). Recent experiments in new-born lambs have shown that pulmonary convertase activity is already present at birth (10). Other experiments on isolated rat (3) and dog (7) lungs have shown that the rate of *ex-vivo* conversion is influenced by modifications in pulmonary blood flow The first purpose of the present study was to find out, using the systemic pressor response technique in children undergoing diagnostic right and left heart catheterization, whether pulmonary conversion in immature humans is similar to that of adults and whether an increased pulmonary blood flow resulting from intracardiac left to-right shunt can modify the degree of this conversion.

The systemic pressor response technique is a method based on the comparison of the height of systemic pressor peaks resulting from alternate administration of a given dose of Angiotensin I into the pulmonary artery and ascending aorta. The technique should give an accurate estimate of pulmonary conversion if two conditions are met (1) Angiotensin has no hemodynamic effect on the pulmonary vasculature that could alter the systemic pressor response, and (2) systemic conversion is negligible as compared to pulmonary conversion, as once suggested by Vane (13 14). It was the second purpose of this study to test the validity of these assumptions. The first was found to be correct and the second was found to be wrong.

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The study was carried out during routine right and left heart catheterization in 16 children. Five had left to right shunts at the atrial level (20% to 60% of the pulmonary blood flow), none having elevated pulmonary vascular resistance. The other 11 patients had no significant hemodynamic abnormalities, they had been investigated to establish the significance of a heart murmur The patients ages ranged from 3 to 12 years. The catheter studies were carried out under light sedation obtained by a mixture of Meperidine, Promethazine and Chlorpromazine.

The systemic pressor response technique was used (2). Catheters were placed into the pulmonary artery and the aortic root, just above the semilunar valves, under fluoroscopic control. The substances used were Asp-Ile$_5$-angiotensin I (A-I) (Schwarz Biochemical) and Asn$_1$-Val$_5$-angiotensin II (Ciba). Dilutions of 1 μg/cc and 10 μg/cc were prepared in advance and kept frozen until used.

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Key words. Mucolipidosis II I cell-phenomenon, lysosomal enzyme depletion, leakage of lysosomal enzymes, mucopolysaccharide metabolism, ultrastructure

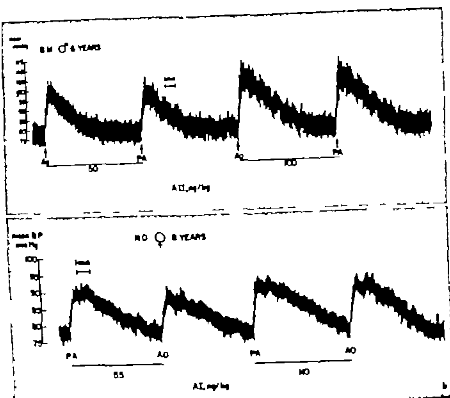


Fig. 2. (*a*) Typical pressor response to two doses of Angiotensin II given alternately into the aortic root (*Ao*) and pulmonary artery (*PA*). For each dose, pressor responses are similar for both routes. The slope of the ascending limb of the responses to either route of injection are virtually similar. (*b*) Pressor responses to two doses of Angiotensin I given alternately into the pulmonary artery and aortic root. Responses to the PA injections are only slightly higher. The pressor potency is 103%. The slope of the ascending limb of responses to PA injections is steeper than that resulting from Ao administration.

With A-I, the pressor potency of the pulmonary artery injection was slightly higher. In children with normal hemodynamics, the potency ratio ranged from 100 to 182% (120± 11.1%). In patients with left-to-right shunts, the pressor potency ratio ranged from 100 to 126% with a mean of 109±4.8%. The difference between subjects with and without a shunt was not statistically significant.

Six patients had a pressor response of comparable magnitude after aortic and PA injections. Fig. 2*b* shows one such case where the calculated pressor potency ratio was 103%.

Pulmonary artery pressor response

In 14 patients, no change in PA pressure was noted after injection of A I or A II given by either route (Fig. 3). In 2 cases was a slight increase in PA pressure noted (2 to 5 mmHg). This occurred later than the systemic pressor response and was obviously secondary to it.

Time course of pressor responses

Intervals were measured from the moment of injection to the onset of the pressor response, and from moment of injection to the time when one half of the maximal pressor response was reached.

With A II, a delay of 4 sec was observed in both measurements after PA injection, as compared with the aortic root injection. This is due to the pulmonary transit time, and has been noted before (3).

With A I the intervals after PA injection were similar to the ones observed for A II.

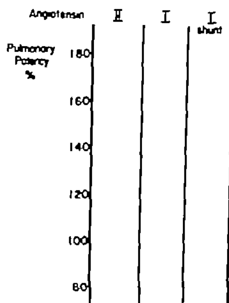


Fig 1 Relative pressor potency of pulmonary injections of Angiotensin II (*left*) Angiotensin I in children without shunts (*middle*) and Angiotensin I in children with left to-right shunts (*right*).

Each child was injected with graded doses of the peptide at 10 min intervals by the aortic route until a pressor response of at least 15 mmHg was obtained This dose and twice this dose called the "low" and the "high" dose respectively were then administered in order to determine the 20 mmHg pressor dose by each route of administration Four injections were made at 15 minute intervals the low dose was given once via the aorta and once via the pulmonary artery the high dose was similarly given once via the aorta and once via the pulmonary artery The order of administration of these injections was made at random so as to cancel the effect of variations in vascular reactivity with time. The rise in mmHg was plotted arithmetically against the logarithm of the dose on semilog paper The dose-response via the pulmonary route was drawn graphically by joining the low-dose response to the high-dose responses the 20 mmHg dose was then found by intrapolation. The same was used to determine the 20 mmHg dose via the aorta The pulmonary-route pressor potency ratio expressed as a percentage, was obtained by the formula.

$$\frac{\text{20 mmHg pressor dose via Ao}}{\text{20 mmHg pressor dose via PA}} \times 100$$

Responses elicited in this study ranged from 10 to 25 mmHg and in no case was any discomfort or side effect observed

RESULTS

Systemic pressor response

Results are shown in Table 1 and summarized in Fig 1

With A II pressor responses were approximately the same for aortic root and pulmonary artery injections. The pressor potency ratio was close to the expected 100% 94± 4 7% (mean and standard error of the mean). (See Fig 2 *a* for an actual example)

Table 1 *Dose required to obtain a 20 mmHg pressor response by pulmonary artery (PA) and aortic root (Ao) injections and pressor potency ratio*

Individual values obtained in 16 children are shown

Group	Patient	Sex	Age	20 mmHg pressor dose via PA (ng/kg)	20 mmHg pressor dose via Ao (ng/kg)	Pulmonary route Pressor potency
A-II no shunt	H S.	M	7	32	26	80
	F R.	M	5	60	60	100
	B. M	M	6	41	39	95
	B. P	M	5	15	15	100
A-I no shunt	S. A	F	12	40	73	182
	Ch. G	M	5	38	49	130
	G A.	M	9	26	31	120
	N J	F	8	150	162	108
	R. S	M	4	55	57	102
	C. L.	F	8	24	24	100
	L M H	F	3	62	62	100
A-I shunt	L. G	M	5	30	38	126
	B. L.	F	7	26	29	110
	J Q	M	8	36	50	108
	L. L.	F	5	83	83	100
	L. P	F	3	60	60	100

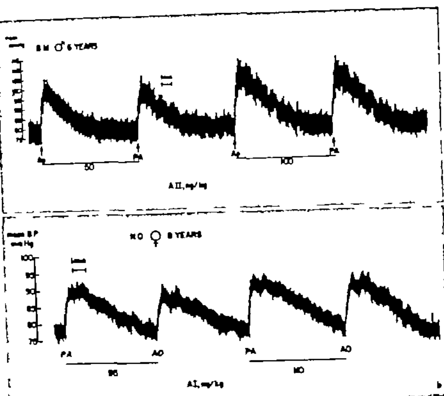


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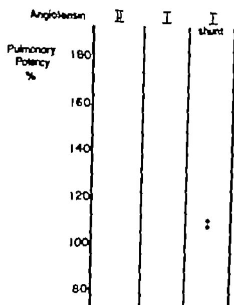


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(). These data challenged the working hypothesis of renin-angiotensin investigators who had usually taken for granted the existence of extrapulmonary conversion, especially in the kidney. Intrarenal conversion was soon to be confirmed by several groups (5 6 8 12). Extrapulmonary conversion as a whole was undeniably demonstrated in vivo by the persistence of pressor responsiveness to A I in dogs without lungs (2) and was also demonstrated to occur *in vitro* (1). Pulmonary conversion itself was eventually found to vary between individuals of the same species (3) when measured with the systemic pressor response assay. This method is based upon the comparison of systemic pressor responses to Angiotensin I injected into the pulmonary artery and ascending aorta alternately. A II is known to cross the lungs without being inactivated in humans (2). Angiotensin I did not alter PA pressure following PA administration. It is therefore unlikely that unwanted pulmonary hemodynamic alterations invalidate our technique, a possibility brought forward by Vane (9).

If the pulmonary vascular bed is the only or major site of conversion of A-I, it should be expected that the injections made into the aorta are followed by a much lesser response than PA injections. In fact, a slight gain in pressor activity by injecting in the PA was observed in 6 of our subjects, while in 6 others, the PA and Ao injections induced similar pressor responses. The latter type of response was reported in some rats and some humans by Biron & Campeau (3) and in all their rats by Kreye & Gross (11). One could postulate that, in those cases, no pulmonary conversion has occurred. The time course data of the pressure responses observed in the present study however invalidate this conclusion. Indeed, the interval from injection to onset of pressor response is similar for A I and A II when these substances are given into the PA. However when A I and A II are injected into the aortic root, both intervals are significantly longer for A I. Conversion in the systemic vascular bed could well be responsible for the delay. Our results can then be explained in the following way: A I injected into the PA is converted to A II and released into the systemic circulation where it acts immediately. On the other hand, A I injected into the aortic root is activated in the systemic circulation (arterioles) where it acts after a delay of 4 sec needed for the conversion. Pulmonary conversion must be a rapid process. It must be completed within less than 1 sec, which is the average pulmonary capillary transit time.

This essentially means that the systemic pressor response technique does not allow evaluation of pulmonary conversion *per se* but only in comparison with systemic conversion. Equal pressor responses would be seen whenever the peripheral conversion is near maximal. It is obvious that, if most of the A I given by the aortic route is converted in systemic vascular beds, there will be no detectable gain in pressor potency by injecting it in the pulmonary artery even if conversion does occur in the lungs. A difference in rapidity of onset of the pressor response is then the only witness of pulmonary conversion. But this difference of about 4 sec is, most likely without biological importance.

Why then would some subjects have an enhancement of the pressor potency by passage of A I through the lungs, whereas others do not? The most likely explanation is in completeness of peripheral conversion. If the latter process converts for example 50% of A I molecules reaching pulmonary passage can at most lead to a 50% gain in pressor activity. This would also mean that all previous work carried out with the systemic pressor response technique deals as much with *peripheral conversion* as with *pulmonary conversion!* Pulmonary conversion in the light of our new findings, would be a constant phenomenon in all preparations, but would be of no physiological importance whenever peripheral conversion is near complete. A greater pulmonary than aortic pressor potency of Angiotensin I is definite evidence of pul-

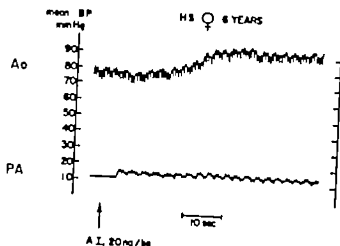


Fig 3 Recording of mean pressures in the aorta (*Ao*) and pulmonary artery (*PA*) after injection of Angiotensin I into the pulmonary artery There is a systemic pressor response but no increase in PA pressure The same could be observed for Angiotensin II

Time to onset was exactly the same time to one half maximal pressor response was slightly but not significantly longer On the other hand after aortic root injection both intervals were significantly longer after A I injection (Table 2)

Fig 4 shows actual tracings taken from one child injected with Angiotensin II (top) and another injected with Angiotensin I (bottom) the latter (C L.) had a pulmonary potency of 100% according to maximal height of pressor peaks.

Table 2. *Time course of the pressor response*

Means and Standard are given in the intervals from injection to onset of pressor response (*t* onset) and from injection to the time when 50% of maximal pressor is reached (*t* ½max.) *P* values were obtained by Student's *t* test NS non-significant

	t onset (sec)		*t* ½max (sec)	
Pulmonary artery				
A-I	6.5	NS	14 9	$p = 0.05$
($N = 10$)	±0.31		±0.75	
A-II	6.2		12.7	
($N = 6$)	±0.28		±0 51	
Aorta				
A-I	6.2	$p < 0.001$	15 7	$p < 0.001$
($N = 10$)	±0.79		±0 72	
A-II	2.2		8 6	
($N = 6$)	±0.52		±0 68	

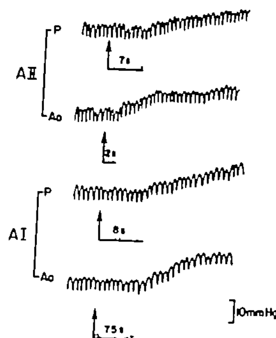


Fig 4 Influence of route of administration on time course of pressor responses in two cases. Tracings show the interval from injection to onset of pressor response For Angiotensin II (case F R.), there was a delay of 5 sec after pulmonary injection due to pulmonary transit time for Angiotensin I (case C L.) the interval between injection and onset was almost similar for both routes, the difference was only of 0.5 sec. If no pulmonary conversion had taken place, the onset of response to the pulmonary injection of A-I should have occurred about 12 sec after injection. The results indicate that pulmonary conversion did occur and yet the maximal heights of pressor peaks resulting from both routes of administration in this case C L. were identical (e g. pulmonary potency = 100%) It must be concluded that pulmonary conversion of A-I takes place undetected by the systemic pressor response technique in the presence of sufficient peripheral conversion in systemic arterioles.

DISCUSSION

The *in vivo* conversion of Angiotensin I to Angiotensin II has received much attention from clinical investigators ever since Ng and Vane showed that A I could double its musculotropic activity during a single passage through the pulmonary circulation of dogs. They claimed that the lung was the major if not only site of conversion in the body (13 14) Biron et al then observed that in some adult humans and some mature laboratory animals, Angiotensin I could double its pressor activity during one pulmonary passage (2, 3

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CLINICAL APPLICATIONS OF ECHOCARDIOGRAPHY IN INFANTS AND CHILDREN

I. *Investigation of Infants and Children without Heart Disease*

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During recent years a rapidly increasing interest has been shown in using pulsed, reflected ultrasound to examine the heart in both adults, infants and children. This investigative technique was formerly termed ultrasoundcardiography but following a suggestion by the American Institute of Ultrasound in Medicine, the name echocardiography has been uniformly adopted.

In infants and children this technique has been used in the study of the mitral valve (15 16), the tricuspid valve (14) the estimation of left or right ventricular size (1 21 26, 27 29), the estimation of left atrial volume (13), the diagnosis of left atrial myxoma (22) and the demonstration of mitral-semilunar valve discontinuity (2). Despite this increasing interest in echocardiography in infants and children, little has been published on the echocardiographic findings in normal infants and children (21 27 29). The aim of the present investigation was to establish echocardiographic data from infants and children without heart disease in order to form a basis for comparison with similar data from infants and children with various forms of heart disease. In addition it was found necessary to test the reproducibility of the echocardiographic measurements.

MATERIAL

Sixty-four infants and children, 33 girls and 31 boys, were examined with echocardiography An attempt was made to examine 4 more children, aged 1 to 3 years, but the examination could not be completed because they were unwilling to cooperate. All the infants and children were hospitalized for disorders not affecting the cardiovascular system. They were all in good general condition, without fever and with a normal heart rate for age. The physical examination of the cardiovascular system was normal and they all had a normal electrocardiogram. The age distribution of the material is presented in Fig. 1 and the distribution of bodyweight in Fig. 2.

In order to test the reproducibility of the method, 11 patients, aged from 2 months to 16 years, were examined with echocardiography on 2 consecutive days. This investigation was performed during a short period of time and the patients, 4 of the above-mentioned children without heart disease and 7 infants and children with various forms of congenital heart disease, were randomly selected from the patients examined with echocardiography during this period.

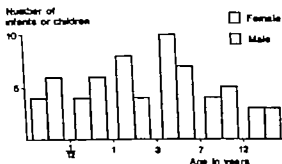


Fig 1 Age and sex distribution of the material.

monary conversion but equal responses by both routes does not rule out pulmonary conversion.

SUMMARY

The systemic pressor response to bolus injections of Angiotensin I (A I) into the pulmonary artery (PA) and the aortic root (Ao) was compared in 11 children during routine left and right heart catheterization five had a left to-right shunt and 6 had no hemodynamic abnormalities. An additional 4 children with normal hemodynamics were tested with Angiotensin II (A II) Systemic pressor peaks to PA injections of A I were slightly higher than those resulting from Ao injections. A study of the time course of the pressor responses showed that the intervals from the injection to onset of response and from injection to half peak were significantly prolonged after injection of A I into the aorta, as compared with injections of A II by the same route Such a difference was not observed when A I and A II were given into the PA. These results indicate that conversion occurs to a considerable degree in the peripheral circulation with a delay that is most probably due to the process of activation When this peripheral conversion is maximal, the systemic pressor response assay is unable to detect pulmonary conversion solely on the basis of the height of pressor peaks, because the responses are equal after PA and Ao injections. PA injections of Angiotensin I and II had no effect on pulmonary artery pressure It is concluded that (1) pulmonary conversion occurs in all children with and without shunt (2) peripheral (systemic) conversion occurs to a considerable degree and can account for most of the overall conversion of Angiotensin I so that the role of the lung in the renin-angiotensin system seems unlikely

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adjustment, an echo from one of the aortic cusps was seen between these two parallel echoes during diastole (Fig. 4). In order to ensure a reproducible transducer position, the transducer was always placed in the third left intercostal space close to the left sternal border and angulated in the superior-medial direction (about 10°–45°) to obtain the two parallel echoes of the aortic root and an echo from an aortic cusp. In 10 of the patients it was not possible to obtain this registration with the transducer in the third intercostal space, but satisfactory recordings could be obtained with the transducer in the second left intercostal space close to the left sternal border and angulated in the same direction or only in the medial direction. In all these recordings an echo-free space was seen between the echo from the posterior wall of the aortic root and an echo positioned further posteriorly. It was suggested by Hirata et al. (12) that this echo-free space represented the left atrium. A confirmation that the echo-giving structures referred to above are in fact the anterior and posterior walls of the aortic root, an aortic cusp and the left atrium has been given by Gramiak et al. (7, 9), using the contrast-echo method. In the registrations made in the present material measurements were made of the distance between the anterior and posterior aortic wall (aortic diameter, AOD, Fig. 4) and the distance between the posterior aortic wall and the posterior wall of the left atrium (left atrial dimension, LAD, Fig. 4). Both these measurements were made at end-systole, defined as the end of the T-wave in the simultaneously recorded electrocardiogram.

Interventricular septum and posterior left ventricular wall

These investigations were performed with the technique described by Popp et al. (24) and further discussed in detail by Feigenbaum (5) and McDonald et al. (20). The transducer was placed in the fourth left intercostal space close to the left sternal border. The transducer was first held in the antero-posterior direction to obtain an echo from the anterior mitral leaflet. When this echo was identified the transducer was angulated slightly in the lateral and caudal direction. The echo from the anterior mitral leaflet was thereby changed into a more faint uncharacteristic echo, but posterior to this the strong echoes from the posterior left ventricular wall appeared. This latter echo has a characteristic anterior movement during ventricular systole (Fig. 5). This strong posterior echo originates presumably from the interface between the pericardium epicardium of the posterior left ventricular wall and the lung tissue. In front of this echo a weaker echo presumably coming from the endocardial posterior wall of the left ventricle, was seen. By adjusting the sensitivity of the ultrasonoscope two parallel echoes could be obtained anterior to the echo from the anterior mitral leaflet and originating from the right and left side of the interventricular septum (Fig. 5).

Between the anterior echo from the interventricular septum (right side of the interventricular septum) and the dense echoes from the chest wall, an echofree space can be seen (Fig. 5). Using the contrast-echo method, Gramiak et al. (9) have shown that this echo-free space is a part of the right ventricle and, using the same method, Feigenbaum et al. (6) have identified the various echo-giving structures in the left ventricle.

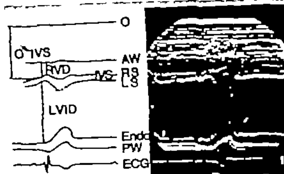


Fig. 5. Echocardiogram of the interventricular septum and the posterior left ventricular wall in a child without heart disease. The sites where measurements were made of left ventricular internal dimension (*LVID*), right ventricular dimension (*RVD*) and the distance between the anterior chest wall and the right side of the interventricular septum (*O-IVS*) are indicated in the line drawing. Abbreviations: *O* = anterior chest wall, *AW* = anterior wall of right ventricle; *RS* = right side of interventricular septum (*IVS*); *LS* = left side of interventricular septum, *Endo* = endocardial posterior wall of left ventricle; *PW* = posterior epicardial wall of left ventricle; *ECG* = electrocardiogram.

Feigenbaum (5) has found that, with the transducer positioned in the fourth left intercostal space at the left sternal border and angulated as suggested above, there is only a very small latitude of aim in which simultaneous echoes from the posterior left ventricular wall and the interventricular septum can be obtained. The same experience has been gained during the present investigation. Due to these special circumstances it is possible to obtain reproducible registrations.

The following measurements were made on the recordings obtained with the technique just described: the distance between the endocardial echo from the posterior left ventricular wall and the echo from the left side of the interventricular septum (left ventricular internal dimension, LVID, Fig. 5), the distance between the two parallel echoes from the interventricular septum (width of the interventricular septum), the distance between the echo from the right side of the interventricular septum and the posterior border of the dense echoes from the anterior chest wall (right ventricular dimension, RVD, Fig. 5) and the distance between the echo from the right side of the interventricular septum and the echo from the anterior surface of the chest wall (O–IVS, Fig. 5). All these measurements were made at end-diastole, defined as the peak of the R-wave in a simultaneously recorded electrocardiogram.

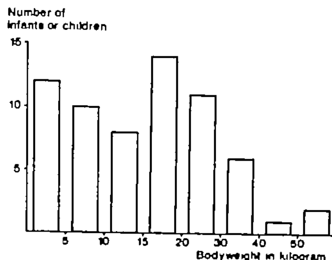


Fig 2 Distribution of body weight of the material.

METHODS

The principles of echocardiography have been thoroughly described earlier (3) The preliminary experiences of the application of this method in infants and children have also been published (17). In the present investigation an ultrasonoscope (Smith Kline Eskoline 20) was used This instrument produces 1 000 pulses/sec and a 2.25MHz transducer of 1 9 cm diameter was used The registrations were made on Polaroid film and a simultaneosly recorded electrocardiogram was used as a reference The patients were examined in the supine position during normal respiration and without premedication. A water-soluble gel was used to obtain airless contact between the transducer and the skin.

In every subject examined reghtrations from the following four regions were made 1 the anterior mitral leaflet 2, the aortic root and the left atrium 3 the interventricular septum and the posterior left ventricular wall, and 4 the anterior tricuspid leaflet.

The anterior mitral leaflet

An echo from the anterior mitral leaflet was obtained with the transducer located at the fourth or sometimes the third left intercostal space 2–5 cm from the midline. The transducer was held in the antero-posterior direction or angulated slightly in the medial or medial-cranial direction. Great care was taken to



Fig 3 Echocardiogram of the anterior mitral leaflet (*AM*) in a child without heart disease. In this and subsequent echocardiograms the top of the figure represents the anterior direction. A schematic line drawing is included. For further explanation see text.



Fig 4 Echocardiogram of the aortic root and the left atrium in a child without heart disease. The sites where the measurements of the aortic root diameter (*AOD*) and the left atrial dimension (*LAD*) were made are indicated in the line drawing.

obtain an echo from the anterior mitral leaflet with as large an amplitude of movement as possible. The echo from the anterior mitral leaflet (Fig. 3) is characterized by a slow anterior movement towards the anterior thoracic wall during ventricular systole (Fig. 3 C–D). In the beginning of diastole a rapid anterior opening movement occurs (Fig. 3 D–E). When the fully open position is reached (Fig. 3 Point E) the movement is reversed and a posterior movement towards a semiopen position takes place (Fig. 3 E–F). The atrial systole causes a new opening (anterior) movement (Fig. 3 A) before a posterior closing movement (Fig. 3 A–C) is seen in the beginning of ventricular systole The speed of movement of the echo from the anterior mitral leaflet in the posterior direction during the early part of diastole (Fig. 3 E–F) before atrial systole was measured whenever possible With a fast heart rate of approximately 120–130/min or above the period of posterior movement before atrial systole is too short to allow valid measurement. Measurements were also made of the total amplitude of movement (vertical distance between points C and E in Fig. 3) and the amplitude of opening movement in the beginning of diastole (vertical distance between points D and E in Fig. 3) of the echo from the anterior mitral leaflet

Aortic root and left atrium

Having obtained a satisfactory echocardiogram from the anterior mitral leaflet the transducer was tilted so that the ultrasonic beam was directed in the superior—medial direction but with the transducer still in the same intercostal space The echo from the anterior mitral leaflet was then gradually changed into an echo with a smaller amplitude of movement when an echo from the mitral ring was obtained Anterior to the echo from the mitral ring an echo could now be obtained with a movement parallel to the movement of the echo from the mitral ring. It has been demonstrated that this echo originates from the inter ventricular septum. By moving the transducer on the anterior chest wall up into the third or second left intercostal space close to the left sternal border and directed in the superior medial direction, two parallel echoes were obtained from the anterior and posterior walls of the aortic root Furthermore with proper

adjustment, an echo from one of the aortic cusps was seen between these two parallel echoes during diastole (Fig. 4). In order to ensure a reproducible transducer position, the transducer was always placed in the third left intercostal space close to the left sternal border and angulated in the superior-medial direction (about 10°–45°) to obtain the two parallel echoes of the aortic root and an echo from an aortic cusp. In 10 of the patients it was not possible to obtain this registration with the transducer in the third intercostal space but satisfactory recordings could be obtained with the transducer in the second left inter costal space close to the left sternal border and angulated in the same direction or only in the medial direction. In all these recordings an echo-free space was seen between the echo from the posterior wall of the aortic root and an echo positioned further posteriorly It was suggested by Hirata et al. (12) that this echo-free space represented the left atrium A confirmation that the echo-giving structures referred to above are in fact the anterior and posterior walls of the aortic root an aortic cusp and the left atrium has been given by Gramiak et al. (7 9), using the contrast-echo method. In the registrations made in the present material measurements were made of the distance between the anterior and posterior aortic wall (aortic diameter AOD Fig. 4) and the distance between the posterior aortic wall and the posterior wall of the left atrium (left atrial dimension, LAD Fig 4). Both these measurements were made at end-systole defined as the end of the T-wave in the simultaneously recorded electrocardiogram.

Interventricular septum and posterior left ventricular wall

These investigations were performed with the technique described by Popp et al. (24) and further discussed in detail by Feigenbaum (5) and McDonald et al. (20). The transducer was placed in the fourth left intercostal space close to the left sternal border The transducer was first held in the anterio-posterior direction to obtain an echo from the anterior mitral leaflet. When this echo was identified the transducer was angulated slightly in the lateral and caudal direction. The echo from the anterior mitral leaflet was thereby changed into a more faint uncharacteristic echo, but posterior to this the strong echoes from the posterior left ventricular wall appeared This latter echo has a characteristic anterior movement during ventricular systole (Fig. 5) This strong posterior echo originates presumably from the interface between the pericardium epicardium of the posterior left entricular wall and the lung tissue. In front of this echo a weaker echo, presumably coming from the endocardial posterior wall of the left entricle was seen. By adjusting the sensitivity of the ultrasonoscope two parallel echoes could be obtained anterior to the echo from the anterior mitral leaflet and originating from the right and left side of the interventricular septem (Fig. 5). Between the anterior echo from the interventricular septum (right side of the interventricular septum) and the dense echoes from the chest wall, an echofree

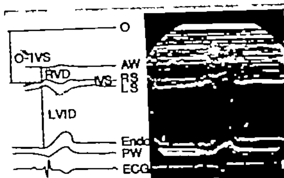


Fig. 5 Echocardiogram of the interventricular septum and the posterior left ventricular wall in a child without heart disease. The sites where measurements were made of left ventricular internal dimension (*LVID*) right ventricular dimension (*RVD*) and the distance between the anterior chest wall and the right side of the interventricular septum (*O-IVS*) are indicated in the line drawing. Abbreviations: *O* – anterior chest wall, *AW* – anterior wall of right ventricle *RS* – right side of interventricular septum (*IVS*) *LS* – left side of interventricular septum; *Endo* – endocardial posterior wall of left ventricle; *PW* – posterior epicardial wall of left ventricle *ECG* – electrocardiogram.

space can be seen (Fig. 5). Using the contrast-echo method, Gramiak et al. (9) have shown that this echo-free space is a part of the right ventricle and, using the same method, Feigenbaum et al. (6) have identified the various echo-giving structures in the left ventricle.

Feigenbaum (5) has found that, with the transducer positioned in the fourth left intercostal space at the left sternal border and angulated as suggested above, there is only a very small latitude of aim in which simultaneous echoes from the posterior left ventricular wall and the interventricular septum can be obtained. The same experience has been gained during the present investigation. Due to these special circumstances it is possible to obtain reproducible registrations.

The following measurements were made on the re cordings obtained with the technique just described. the distance between the endocardial echo from the posterior left ventricular wall and the echo from the left side of the interventricular septum (left ventric ular internal dimension, LVID Fig. 5) the distance between the two parallel echoes from the interventric ular septum (width of the interventricular septum), the distance between the echo from the right side of the interventricular septum and the posterior border of the dense echoes from the anterior chest wall (right ventricular dimension, RVD Fig. 5), and the distance between the echo from the right side of the inter ventricular septum and the echo from the anterior surface of the chest wall (O-IVS, Fig. 5). All these measurements were made at end-diastole defined as the peak of the R-wave in a simultaneously recorded electrocardiogram.

Table 1 *Reproducibility of the echocardiographic measurements*

Abbreviations AOD = aortic root diameter· LAD = left atrial dimension, LVID = left ventricular internal dimension O–IVS = distance between anterior chest wall and the right side of interventricular septum RVD = right ventricular dimension AM ampl. = amplitude of movement of echo from the anterior mitral leaflet

		95% confidence interval (mm)
AOD	mean of 10 measurements	±1.2
	mean of 5 measurements	±1.3
LAD	mean of 10 measurements	±2.6
	mean of 5 measurements	±2.7
LVID	mean of 10 measurements	±1 9
	mean of 5 measurements	±2.0
O–IVS	mean of 10 measurements	±2.8
	mean of 5 measurements	±3 0
RVD	mean of 10 measurements	±1 1
	mean of 5 measurements	±1.2
AM ampl.	mean of 10 measurements	±1.5
	mean of 5 measurements	±1 9

The anterior tricuspid leaflet

After the echoes from the interventricular septum had been identified a search was made for the echo from the anterior tricuspid leaflet This echo has a pattern of movement identical to that from the anterior mitral leaflet (Fig. 3) The transducer was placed in the fourth left intercostal space close to the left sternal border The transducer had to be angulated 10°–45 in the medial direction to obtain the characteristic echo from the anterior tricuspid leaf let. In some of the infants a complete echo could be obtained but in the other infants and in all the children only an echo with a slow anterior movement dur ing ventricular systole and a rapid anterior opening movement in the beginning of diastole was seen Dur ing the remaining part of the heart cycle no echo could be recorded. When possible the same measurements as those for the echo from the anterior mitral leaflet were made.

Test of reproducibility of the method

In all the patients included in this part of the investigation a complete echo-cardiographic examination as outlined above was performed. Enough recordings were obtained to allow 10 measurements of all the data required The following day the same procedure was repeated. The mean difference in heart rate between these two investigations was 5 4% for all the patients, with a range of 0.9%–12% The re sult of this part of the study (see below) showed that the accuracy of the method increased only slightly when a mean of 10 measurements was used instead of a mean of 5 measurements. Except in the analysis of the reproducibility of the measurement a mean value of five measurements was therefore used in this study

For the statistical analysis, standard statistical methods were used.

RESULTS

In all the results presented below no difference was found between girls and boys. The results are therefore presented for the whole material

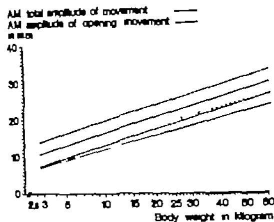
Reproducibility of echocardiographic measurements

A statistical analysis was made with a comparison of the results of the various measurements made on the first and the second day This comparison was made both with the results given as a mean of 10 measurements and as a mean of 5 measurements. The result of this comparison is expressed as a 95% confidence interval of the measurement and is shown in Table 1

If the standard deviations used in these confidence intervals are divided by the actual measurements (mean of 10 measurements) one obtains a coefficient of variation which can be used as a measurement of reproducibility For all these measurements this coefficient of variation lies between 1% and 7% When a mean of 5 measurements was used instead of 10 measurements the coefficient of variation increased by about 1% (i.e. from 2 to 3%). Thus the accuracy of the method increased only slightly on using a mean of 10 instead of a mean of 5 measurements. The latter method was therefore used for the rest of this investigation

Echocardiography of anterior mitral leaflet

A satisfactory recording of the echo from the anterior mitral leaflet could be obtained in all the subjects examined. The results of the measurement of the total amplitude of movement and the amplitude of opening movement in the beginning of diastole showed a linear correlation to the height of the patients, and to the cube root of the body weight and to the



...g. 6. Nomogram for prediction of total amplitude ... movement and amplitude of opening movement of ... echo from the anterior mitral leaflet (*AM*) based ... body weight in infants and children without heart ...ease. This nomogram is based on the linear correla... between these amplitudes and the cube root of ... body weight (AM total amplitude of movement ...=7.56 x+0.17 r=0.95 S.D 1.62; AM amplitude ... opening movement. y=6.68 x−2.35 r=0.95 S.D ...44). The regression lines and the 95% prediction ...tervals are indicated.

...ube root of the age. Since the best correla...ion was to the cube root of the body weight, ... nomogram was constructed based on this ...orrelation (Fig. 6)

The speed of movement of the echo from ...terior mitral leaflet in the posterior direc...ion during the early part of diastole could ...e measured in 57 of the 64 subjects examined. The 7 individuals in whom this was not pos...sible were all infants less than 1 year of age, with too rapid a heart rate to allow a *reliable* measurement.

For the whole material the range of this speed of movement was 90–180 mm/sec, mean value 130 mm/sec. There was no clear correlation between this speed of movement and age, height or weight. The group of 13 infants below 1 year of age did, however have a somewhat lower speed of movement (range 90–130 mm/sec, mean 105 mm/sec).

Echocardiography of aortic root-left atrium

A satisfactory recording of the echoes from the aortic root and the left atrium was ob-

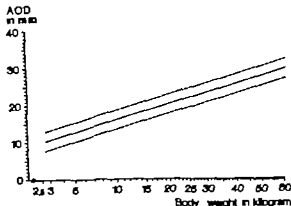


Fig. 7. Nomogram for prediction of aortic root diameter (*AOD*) on echocardiogram based on body weight in infants and children without heart disease. This nomogram is based on the linear correlation between AOD and the cube root of the body weight (y=7.48 x−0.10 r=0.97 S.D 1.24). Regression line and 95% prediction interval are indicated.

tained in all the subjects examined. In 54 of the infants and children this registration could be made with the transducer located in the third left intercostal space at the left sternal border and in the remaining 10 the transducer was placed in the second left intercostal space instead. The measurements of the aortic diameter (AOD) and the left atrial dimension (LAD) showed a linear correlation to the height, and to the cube root of the body

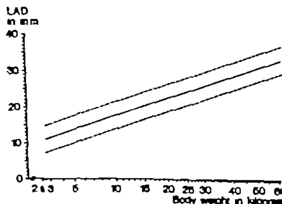


Fig. 8. Nomogram for prediction of left atrial dimension (*LAD*) on echocardiogram based on body weigh... in infants and children without heart disease. Thi... nomogram is based on the linear correlation betwee... LAD and the cube root of the body weight (y=8.4... x−0.60 ... =0.94 S.D 1.89). Regression line and 95% prediction interval are indicated.

Table 1 *Reproducibility of the echocardiographic measurements*

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O-IVS	mean of 10 measurements	±2.8
	mean of 5 measurements	±3.0
RVD	mean of 10 measurements	±1 1
	mean of 5 measurements	±1.2
AM ampl	mean of 10 measurements	±1.5
	mean of 5 measurements	±1.9

The anterior tricuspid leaflet

After the echoes from the interventricular septum had been identified a search was made for the echo from the anterior tricuspid leaflet This echo has a pattern of movement identical to that from the anterior mitral leaflet (Fig. 3) The transducer was placed in the fourth left intercostal space close to the left sternal border The transducer had to be angulated 10°–45 in the medial direction to obtain the characteristic echo from the anterior tricuspid leaflet. In some of the infants a complete echo could be obtained, but in the other infants and in all the children only an echo with a slow anterior movement during ventricular systole and a rapid anterior opening movement in the beginning of diastole was seen. During the remaining part of the heart cycle no echo could be recorded. When possible the same measurements as those for the echo from the anterior mitral leaflet were made

Test of reproducibility of the method

In all the patients included in this part of the investigation a complete echo-cardiographic examination as outlined above was performed. Enough recordings were obtained to allow 10 measurements of all the data required. The following day the same procedure was repeated The mean difference in heart rate between these two investigations was 5 4% for all the patients, with a range of 0.9%–12% The result of this part of the study (see below) showed that the accuracy of the method increased only slightly when a mean of 10 measurements was used instead of a mean of 5 measurements. Except in the analysis of the reproducibility of the measurement a mean value of five measurements was therefore used in this study

For the statistical analysis, standard statistical methods were used

RESULTS

In all the results presented below no difference was found between girls and boys. The results are therefore presented for the whole material

Reproducibility of echocardiographic measurements

A statistical analysis was made with a comparison of the results of the various measurements made on the first and the second day This comparison was made both with the results given as a mean of 10 measurements and as a mean of 5 measurements. The result of this comparison is expressed as a 95% confidence interval of the measurement and is shown in Table 1

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Echocardiography of anterior mitral leaflet

A satisfactory recording of the echo from the anterior mitral leaflet could be obtained in all the subjects examined. The results of the measurement of the total amplitude of movement and the amplitude of opening movement in the beginning of diastole showed a linear correlation to the height of the patients, and to the cube root of the body weight and to the

The right ventricular dimension (RVD) did not show any appreciable correlation with age, height or weight. Only small variations were found in these measurements. The mean value of RVD for the entire normal material was 7.3 mm with a 95% prediction interval of 4.3 to 10.3 mm.

Echocardiography of anterior tricuspid leaflet

An echo from the anterior tricuspid leaflet could be obtained in all infants and children. A complete echo, however could only be recorded in the youngest infants (5 of 12 infants below 3 months of age). The total amplitude of movement was equal to or up to 2 mm larger than the total amplitude of movement of the anterior *mitral* leaflet for the same individual. A satisfactory estimation of the speed of movement during diastole could not be obtained in any of the cases.

For the rest of the infants and all children the echo from the anterior tricuspid leaflet consisted of an echo seen during ventricular systole with a slow anterior movement and a rapid anterior opening movement in the beginning of diastole identifying the echo-giving structure as an atrioventricular valve. Since this echo was obtained anterior to the echo from the interventricular septum and more to the right, confusion with an echo from the anterior *mitral* leaflet was easily avoided.

DISCUSSION

Reproducibility of echocardiographic measurements

In reports published about the use of echocardiography in infants and children, no information seems to have been presented about the reproducibility of the echocardiographic measurements. Pombo et al. (23) have analysed the reproducibility of echocardiographic measurements of left ventricular dimensions and volumes in adults. The measurement of the left ventricular internal dimension in end-diastole (LVID) in the material presented by Pombo et al. (23) is directly comparable to the measurement of LVID in the present material. A calculation of the coefficient of variation of the measurements reported by Pombo et al. (23) of LVID made by two observers from the same echocardiogram gives a value of 1–3%. The same authors also compared measurements made by two observers from different echocardiograms of the same patients at different times. A calculation of the coefficient of variation from these data gives a value of 4–6%. The corresponding coefficient of variation of measurement of LVID in the present material made by one observer but on different echocardiograms from the same patients at different times is 2–5%. In the present material the echocardiograms were not examined by two observers but the data given by Pombo et al. (23) and referred to above indicate that the difference in results between two observers examining one echocardiogram is smaller than the difference between two different examinations of the same patient.

Gustafson (10) has made a detailed analysis on adults of the reproducibility of the echocardiographic measurements from the anterior mitral leaflet. For the total amplitude of movement of the echo from the anterior mitral leaflet he found a coefficient of variation of 2–4% during continuous recording and about 6% for measurements made at discontinuous recordings. The corresponding value in the present material is 3–7%.

The reproducibility of the echocardiographic measurements in the present material thus seems to agree rather well with the few reported studies on this matter.

Echocardiography of the anterior mitral leaflet

Echocardiographic findings from the anterior mitral leaflet in infants and children without heart disease has, to the best of our knowledge, only been reported by Meyer et al. (21) and then only from the neonatal period. They found a total amplitude of movement of 6–12

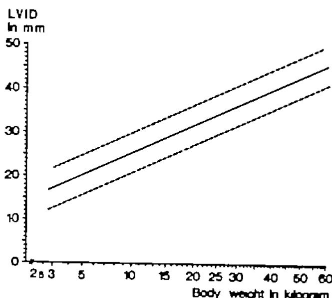


Fig 9 Nomogram for prediction of left ventricular internal dimension (*LVID*) on echocardiogram based on body weight in infants and children without heart disease. This nomogram is based on the linear correlation between LVID and the cube root of the body weight ($y = 11.59\ x + 0.45$ $r = 0.95$ S.D 2.33) Regression line and 95% prediction interval are indicated

weight and to the cube root of the age Since the best correlation was to the cube root of the body weight, nomograms were constructed for prediction of aortic root diameter (AOD)

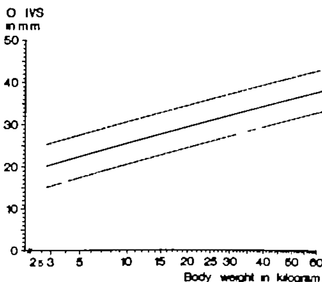


Fig 10 Nomogram for prediction of the distance between the anterior chest wall and the right side of the interventricular septum (*O-IVS*) on echocardiogram based on body weight in infants and children without heart disease. This nomogram is based on the linear correlation between O-IVS and the cube root of the body weight ($y = 7.36\ x + 9.94$ $r = 0.88$ S.D 2.55) Regression line and 95% prediction interval are indicated.

and left atrial dimension (LAD) based on body weight The normal values for these measurements are given as such nomograms (Figs. 7 and 8)

Echocardiography of interventricular septum and posterior left ventricular wall

In all the subjects examined, satisfactory echoes could be obtained from the posterior left ventricular wall including its endocardial surface and the interventricular septum. An echofree space anterior to the echo from the interventricular septum could be seen in all individuals. The anterior margin of this echo-free space was, however not always distinct and the measurement of the right ventricular dimension (RVD) was therefore taken as the largest echo-free space between the echo from the anterior (right) side of the interventricular septum and the posterior margin of the dense echoes from the anterior chest wall

In all subjects two parallel echoes from the interventricular septum could be seen These echoes moved in a posterior direction towards the echo from the posterior left ventricular wall, during ventricular systole The distance between the two parallel echoes (width of the interventricular septum) was in infants and children with a body weight less than 10 kg between 3 and 5 mm In children with a body weight between 10 and 25 kg between 4 and 6 mm and in children with a body weight greater than 25 kg between 5 and 7 mm

The left ventricular internal dimension (LVID) and the distance between the echo from the anterior (right) side of the interventricular septum and the echo from the anterior surface of the chest wall (O-IVS) both showed a linear correlation to the height, to the cube root of the body weight and to the cube root of age The best correlation for both these distances was to the cube root of the body weight and the results are therefore presented as nomograms based on these correlations (Figs. 9 and 10)

due to some difference in the recording technique. The anterior border of the echofree space representing the right ventricle has been found difficult to delineate distinctly in some cases. For this reason another approach was tried. the distance from the echo from the anterior surface of the chest wall to the echo from the anterior (right) side of the inter ventricular septum was measured. To test the applicability of these measurement a comparison between echocardiographic estimation and angiocardiographic estimation of left and right ventricular size has been made and will be reported (19).

Limiting factors and risks involved in echocardiography

An echocardiographic examination could be performed on most of the infants and children examined. The nervous children would also presumably have cooperated in the ex amination under slight sedation but this was not considered to be justified in infants and children without heart disease.

Deformation of the anterior chest wall and interposition of lung tissue in front of the heart, two factors limiting the possibility of an echocardiographic examination, were not encountered in the present material.

The potential risks of the echocardiographic examination have been discussed by several authors (3, 11 25 30) No complications have ever been described on using ultrasound applied as pulsed reflected echocardiography It is also considered unlikely that any complications will occur because of the wide margin of safety or as expressed by Woodward et al. (30) in their conclusion, "a danger threshold of exposure is likely to be very much higher than that commonly used during clinical ultrasonograms"

SUMMARY

Echocardiographic examinations have been performed on 64 infants and children without heart disease, aged from 3 days to 15 years. Examinations of the following four regions were made 1 the anterior mitral leaf let 2, the aortic root and the left atrium 3 the interventricular septum and the posterior left ventricular wall, and 4 the anterior tricuspid leaflet.

A test of the reproducibility of the echocardiographic measurements was performed by examining eleven subjects on 2 consecutive days. Four of these 11 infants and children had no signs of heart disease while the other 7 had various forms of congenital heart disease.

The results of the investigation of infants and children without heart disease are presented as nomograms for the prediction of various echocardiographic measurements in relation to the body weight. Such nomograms are presented for the amplitude of movement of the echo from the anterior mitral leaflet, the aortic root diameter a left atrial dimension, a left ventricular internal dimension and the distance between the anterior chest wall and the right side of the interventricular septum. Normal values are also given for a right ventricular dimension and the width of the interventricular septum.

ACKNOWLEDGEMENTS

The statistical analysis was performed in collaboration with Holger Rootzén, Civ.ing., Department of Mathematical Statistics, University of Lund.

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mm and a speed of movement of 34–56 mm/sec In the present material the corresponding values are 10–13 mm and 90–130 mm/sec respectively There is no obvious explanation for this discrepancy Gustafson (10) has, however pointed out the influence of the total amplitude of movement on the speed of movement. The highest speed of movement was found in registrations with the largest total amplitude of movement. In the present material a very careful search was therefore made to obtain an echo from the anterior mitral leaflet with as large a total amplitude of movement as possible.

A comparison between values for the total amplitude of movement and for the speed of movement during diastole of the echo from the anterior mitral leaflet in normal adults presented by Edler (4) and the corresponding values obtained from the oldest children in the present material show a very good agreement.

The normal values presented here are naturally intended to be used as references for children with heart disease Deviations from the normal in this respect have been found in congenital mitral stenosis (16) and mitral atresia and related disorders (15)

Echocardiography of the aortic root and the left atrium

To our knowledge reports concerning studies on the aortic root by echocardiography in infants and children have not been published previously Normal values for the aortic root diameter in adults estimated by echocardiography have been given by Gramiak et al (8) The mean value of the aortic root diameter in adults is approximately 6 mm above the mean value for the oldest children presented here. This difference seems reasonable since further growth can be expected for the oldest children presented in this material An estimation of left atrial volumes by echocardiography in infants and children has been reported by Hirata et al. (13) but normal values were not given In the study by Meyer et al. (21) of echocardiography in the neonate, normal values for the left atrial dimension were 6–13 mm which is a larger range than that measured in the youngest infants in the present material (9–13 mm) but otherwise shows a good agreement.

A comparison between the estimation of the aortic root diameter (AOD) and left atrial dimension (LAD) by echocardiography and the estimation of the size of the aortic root and the left atrium by angiocardiography in a large material of infants and children has been undertaken and will be reported (18)

Echocardiography of the interventricular septum and the posterior left ventricular wall

The pattern of movement of the echo from the interventricular septum found in the present material is in agreement with that observed in normal adults and described in detail by McDonald et al. (20) The width of the interventricular septum has been measured in adults by Troy et al (28) using the same technique as in the present investigation. Normal values were not reported however A measurement of the left ventricular internal dimension by echocardiography in neonates has been made by Meyer et al. (21) and Winsberg (29). The normal values given in these reports (12–20 mm and 16–20 mm respectively) agree well with the corresponding values of 13–19 mm in the present material. An estimation of the right ventricular size by echocardiography in infants and children has been attempted by Meyer et al (21) in neonates and by Tajik et al (27) in various age groups. In normal neonates Meyer et al. (21) found a right ventricular dimension (RVD) of 10–17 mm Tajik et al. (26) found the corresponding values in infants and children with a body surface area less than 1 m^2 to be 4–14 mm and for larger children 7–14 mm. In the present material the right ventricular dimension (RVD) was 4–11 mm, with no appreciable correlation to age. Th

due to some difference in the recording technique. The anterior border of the echofree space representing the right ventricle has been found difficult to delineate distinctly in some cases. For this reason another approach was tried, the distance from the echo from the anterior surface of the chest wall to the echo from the anterior (right) side of the inter ventricular septum was measured. To test the applicability of these measurement a comparison between echocardiographic estimation and angiocardiographic estimation of left and right ventricular size has been made and will be reported (19).

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SUMMARY

Echocardiographic examinations have been performed on 64 infants and children without heart disease, aged from 3 days to 15 years. Examinations of the following four regions were made. 1 the anterior mitral leaflet, 2, the aortic root and the left atrium 3 the interventricular septum and the posterior left ventricular wall, and 4 the anterior tricuspid leaflet.

A test of the reproducibility of the echocardiographic measurements was performed by examining eleven subjects on 2 consecutive days. Four of these 11 infants and children had no signs of heart disease while the other 7 had various forms of congenital heart disease.

The results of the investigation of infants and children without heart disease are presented as nomograms for the prediction of various echocardiographic measurements in relation to the body weight. Such nomograms are presented for the amplitude of movement of the echo from the anterior mitral leaflet, the aortic root diameter a left atrial dimension, a left ventricular internal dimension and the distance between the anterior chest wall and the right side of the interventricular septum. Normal values are also given for a right ventricular dimension and the width of the interventricular septum.

ACKNOWLEDGEMENTS

The statistical analysis was performed in collaboration with Holger Rootzén, Civ.ing., Department of Mathematical Statistics, University of Lund.

This work was supported by a grant from the Swedish National Association against Heart and Chest Diseases.

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mm and a speed of movement of 34–56 mm/sec In the present material the corresponding values are 10–13 mm and 90–130 mm/sec respectively There is no obvious explanation for this discrepancy Gustafson (10) has, however pointed out the influence of the total amplitude of movement on the speed of movement. The highest speed of movement was found in registrations with the largest total amplitude of movement In the present material a very careful search was therefore made to obtain an echo from the anterior mitral leaflet with as large a total amplitude of movement as possible

A comparison between values for the total amplitude of movement and for the speed of movement during diastole of the echo from the anterior mitral leaflet in normal adults presented by Edler (4) and the corresponding values obtained from the oldest children in the present material show a very good agreement.

The normal values presented here are naturally intended to be used as references for children with heart disease Deviations from the normal in this respect have been found in congenital mitral stenosis (16) and mitral atresia and related disorders (15)

Echocardiography of the aortic root and the left atrium

To our knowledge reports concerning studies on the aortic root by echocardiography in infants and children have not been published previously Normal values for the aortic root diameter in adults estimated by echocardiography have been given by Gramiak et al. (8) The mean value of the aortic root diameter in adults is approximately 6 mm above the mean value for the oldest children presented here. This difference seems reasonable since further growth can be expected for the oldest children presented in this material. An estimation of left atrial volumes by echocardiography in infants and children has been reported by Hirata et al. (13) but normal values were not given In the study by Meyer et al. (21) of echocardiography in the neonate normal values for the left atrial dimension were 6–13 mm which is a larger range than that measured in the youngest infants in the present material (9–13 mm) but otherwise shows a good agreement

A comparison between the estimation of the aortic root diameter (AOD) and left atrial dimension (LAD) by echocardiography and the estimation of the size of the aortic root and the left atrium by angiocardiography in a large material of infants and children has been undertaken and will be reported (18)

Echocardiography of the interventricular septum and the posterior left ventricular wall

The pattern of movement of the echo from the interventricular septum found in the present material is in agreement with that observed in normal adults and described in detail by McDonald et al. (20). The width of the interventricular septum has been measured in adults by Troy et al. (28) using the same technique as in the present investigation. Normal values were not reported however A measurement of the left ventricular internal dimension by echocardiography in neonates has been made by Meyer et al. (21) and Winsberg (29) The normal values given in these reports (12–20 mm and 16–20 mm respectively) agree well with the corresponding values of 13–19 mm in the present material. An estimation of the right ventricular size by echocardiography in infants and children has been attempted by Meyer et al. (21) in neonates and by Tajik et al. (27) in various age groups. In normal neonates Meyer et al. (21) found a right ventricular dimension (RVD) of 10–17 mm. Tajik et al. (26) found the corresponding values in infants and children with a body surface area less than 1 m² to be 4–14 mm and for larger children 7–14 mm. In the present material the right ventricular dimension (RVD) was 4–11 mm with no appreciable correlation to age This discrepancy may be

due to some difference in the recording technique. The anterior border of the echofree space representing the right ventricle has been found difficult to delineate distinctly in some cases. For this reason another approach was tried, the distance from the echo from the anterior surface of the chest wall to the echo from the anterior (right) side of the interventricular septum was measured. To test the applicability of these measurement a comparison between echocardiographic estimation and angiocardiographic estimation of left and right ventricular size has been made and will be reported (19)

Limiting factors and risks involved in echocardiography

An echocardiographic examination could be performed on most of the infants and children examined. The nervous children would also presumably have cooperated in the examination under slight sedation but this was not considered to be justified in infants and children without heart disease.

Deformation of the anterior chest wall and interposition of lung tissue in front of the heart, two factors limiting the possibility of an echocardiographic examination, were not encountered in the present material.

The potential risks of the echocardiographic examination have been discussed by several authors (3 11 25 30). No complications have ever been described on using ultrasound applied as pulsed reflected echocardiography It is also considered unlikely that any complications will occur because of the wide margin of safety or as expressed by Woodward et al. (30) in their conclusion. a danger threshold of exposure is likely to be very much higher than that commonly used during clinical ultrasonograms"

SUMMARY

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CLINICAL APPLICATIONS OF ECHOCARDIOGRAPHY IN INFANTS AND CHILDREN

II. *Estimation of Aortic Root Diameter and Left Atrial Size A Comparison between Echocardiography and Angiocardiography*

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In pediatric cardiology there will always be an interest in using non-invasive methods for the investigation of patients. In recent years this has been reflected in the various publications on echocardiography in infants and children (5, 18, 22, 23 25 26, 28, 29) Echocardiography is well suited for distance measurements and this technique has been used in adults for studies of the aortic root (11 13 16). The possibility of using ultrasound for the estimation of left atrial size was first suggested by Hirata et al. (17) and has also been applied in studies on infants and children (18).

In a previous investigation from this laboratory (24) it was shown that echocardiographic intracardiac distance measurements can be performed in infants and children and that the results were reproducible. The aim of the present investigation was to evaluate the use of echocardiography for measuring the aortic root diameter and the left atrial size by comparing it with the results obtained at angiocardiography

MATERIAL

During a period of 18 months, an echocardiographic examination was performed on every infant and child undergoing heart catheterization and angiocardiography From the angiocardiographic examinations, measurements of the aortic root diameter as defined below could be made in 166 cases. Of these, some patients were excluded for comparison with echocardiography due to the following reasons. (1) Failure to obtain an echocardiographic examination. In one patient, 3 months old, with ventricular septal defect and a severe obstructive bronchitis with hyperinflation of the lungs it was impossible to get any echoes at all from the heart, presumably because there was lung tissue in front of the heart. (2) Malposition of the heart. In 2 patients cardiac echoes could not be obtained in the usual area. One of these patients had a complete situs inversus and the other a dislocation of the heart due to a mediastinal tumour (3) Malposition of the aorta. Since the echocardiographic measurements are based on a normal position of the aorta, all patients with malposition of the aorta had to be excluded. For this reason the following patients were excluded. 8 patients with transposition of the great arteries, 3 patients with corrected transposition of the great arteries with ventricular inversion, 1 patient with double outlet right ventricle, and 3 patients with truncus arteriosus communis. Comparison between echocardiographic and angiocardiographic measurements of the aortic root diameter could thus be made in 148 patients. The age distribution of these patients is shown in Fig. 1

From the original material a measurement of a left atrial dimension by angiocardiography as defined below could be made in 91 patients. Some of these patients had to be excluded for the following reasons. (1) Failure to obtain an echocardiographic examination. This applied to the same patient as described above. (2) Malposition of the heart. Even these were the 2 patients described earlier (3) Malposition of the aorta. The echocardiographic examination of the left atrium is based on its close relation to a normally positioned aortic root. Malposition of the aorta excluded 7 patients with transposition of the great arteries, 1 patient with double outlet right ventricle and 1 patient with corrected transposition of the great arteries with ventricular inversion. Comparison between echocardiographic and angiocardiographic estimations of a left atrial dimension could thus be made in 79 patients. The age distribution of these patients is shown in Fig. 2.

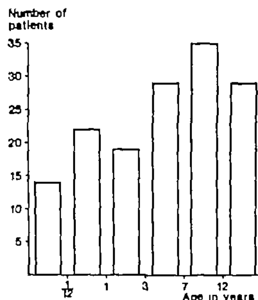


Fig 1 Age distribution of the material where a comparison was made between an aortic root diameter (*AOD*) measured by echocardiography and angiocardiography

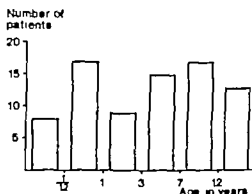


Fig 2 Age distribution of the material where a comparison was made between a left atrial dimension (*LAD*) measured by echocardiography and angiocardiography

METHODS

Echocardiographic examination

In most cases the echocardiographic examination was performed the day before the angiocardiographic examination. The patients were examined without premedication. In a few nervous children between 1 and 3 years of age it was not possible however to obtain satisfactory registrations in this way. These patients were reexamined some hours after the angiocardiography while still under the influence of the premedication given for the angiocardiographic examination. In this way satisfactory recordings were obtained in all patients with exception of one patient referred to above. The patients were examined in the supine position and during normal respiration. The principles of echocardiography have been thoroughly described earlier (7) and a detailed presentation of the technique used in this study has been published recently (24). A commercially available ultrasonoscope (Smith Kline Eskoline 20) was used. This instrument has a repetition rate of 1 000 pulses/sec and 2.25 MHz transducer of 1.9 cm diameter was used. A water-soluble gel was used to obtain airless contact between the transducer and the skin. The registrations were made on Polaroid film and a simultaneously recorded electrocardiogram served as a reference.

A detailed description of the technique used in this material to obtain echoes from the aortic root and the left atrium has been reported recently (24). Two prerequisites were considered necessary in order to obtain satisfactory recordings with reproducible measurements. (1) The transducer should be placed in the third or second left intercostal space close to the left sternal border and angulated in the superior medial direction to obtain the two parallel echoes of the aortic root and behind them the echofree space representing the left atrium (Fig. 3), (2) an echo from an aortic leaflet should be obtained between the two parallel echoes from the aortic root (Fig 3). In 3 neonates it was impossible to obtain any echoes from the aortic leaflet. These infants were shown to have the narrowest aortic roots in the whole material. These three patients died, and the diagnosis of aortic valve atresia was confirmed in all of them.

In the registrations of the echocardiogram from the aortic root/left atrium, measurements were made of the distance between the anterior and posterior wall of the aortic root (aortic root diameter *AOD*, Fig. 3) and of the distance between the posterior wall

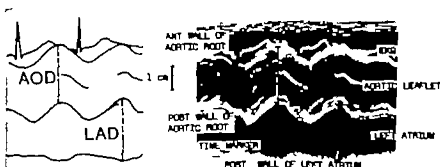


Fig 3 Echocardiogram of the aortic root and the left atrium. The top of the figure represents the anterior direction. The schematic drawing indicates the sites where the measurements of the aortic root dimension (*AOD*) and the left atrial dimension (*LAD*) were made.



Fig. 4. Angiocardiogram of the aortic root in lateral projection. The site where the measurements of the aortic root diameter was made is indicated.

of the aortic root and the posterior wall of the left atrium (left atrial dimension, LAD Fig. 3). Both these measurements were made in end-systole defined as the end of the T-wave in the simultaneously recorded electrocardiogram. For comparison with the angiocardiographic measurements, a mean of 5 echocardiographic measurements was used. All measurements were made by one and the same person. The reproducibility of the echocardiographic measurements has been reported earlier (24).

Angiocardiographic examination

The heart catheterization and angiocardiographic examination were performed without general anaesthesia, the procedure followed that generally employed at this department (2). Infants and children more than 3 months of age received premedication with a mixture of Demerol®, Largactil® and Phenergan® while infants below 3 months of age received no premedication. The radiological measurements were made on full size angiocardiograms. The requirements of the angiocardiogram used in this study were that the left atrium or the aortic root was clearly outlined irrespective of where the contrast injection was made. Measurements of the aortic root diameter were made on angiocardiograms in the lateral projection. The antero-posterior diameter was measured just above bulbus aortae (Fig. 4). In the angiocardiograms of the left atrium made in the lateral projection, the largest sagittal diameter was measured (Fig. 5). The measurements were made at the end of ventricular systole. The focus-film distance was 100 cm. The distance between the aortic root/left atrium and the roentgen film was practically the same (26–27 cm) in the lateral projection irrespective of the size of the patient. The magnification factor in the lateral projection was therefore practically the same (1.35–1.37) in all patients. A correction for the magnification was therefore not made.

RESULTS

The results of the measurements of the aortic root dimension by echocardiography (AOD echo) compared with the corresponding aortic root diameter measured on the angiocardiogram (AOD angio) are given in Fig. 6 for the entire material. As can be seen, there is a very close linear correlation between these two



Fig 5 Angiocardiogram of the left atrium in lateral projection. The site where the measurements of the left atrial diameter was made is indicated.

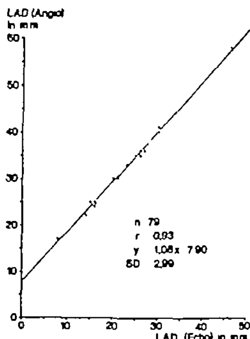


Fig 7 A comparison between the left atrial dimension measured by echocardiography (*LAD Echo*) and the left atrial sagittal diameter measured on the angiocardiogram (*LAD Angio*) in the entire material. The regression line is indicated.

measurements over a wide range of values. The angiographic measurements are slightly larger than the echocardiographic measurements This discrepancy is not surprising since a correction for the magnification of the angiocardiographic measurements was not made

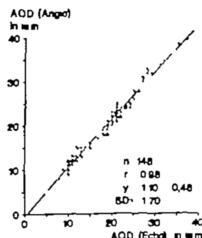


Fig 6 A comparison between the aortic root dimension measured by echocardiography (*AOD Echo*) and the aortic root diameter measured on the angiocardiogram (*AOD Angio*) in the entire material The regression line is indicated

The results of the measurements of the left atrial dimension by echocardiography (LAD echo) compared with the corresponding left *atrial diameter measured on the angiocardiogram* (LAD angio) are given in Fig. 7 The angiographic measurements are larger than those from echocardiography This is not surprising since the angiographic measurements are made as the antero-posterior diameter and the echocardiographic measurements are performed in an oblique direction Besides a correction for the magnification of the angiocardiographic measurements was not made.

DISCUSSION

Examination of the aortic root

The possibility of examining the aortic valve and the outflow tract of the left ventricle by echocardiography was first suggested by Edler (7 8) An *in vivo* confirmation that the actual echoes did originate from the aortic root was given by Gramiak et al. (12) using the con-

trast-echo method. Further studies in adults of the aortic root by echocardiography have also been published (11 13 16) The aortic root diameter in adults with various forms of heart disease has been presented (13) but a comparison between echocardiographic and angiocardiographic measurements of the aortic root diameter does not seem to have been published previously Since the reproducibility of these measurements has been found to be reasonably good (24), the echocardiographic examination of the aortic root can provide additional information about a patient with heart disease.

The 3 patients with the smallest aortic root diameter in the present material (Fig. 6) can be used as good examples of the usefulness of this information. The aortic root diameter in these 3 patients was clearly below the lower normal border (24) This observation together with the echocardiographic findings from the mitral valve region (23) provided very strong suspicion that these patients had aortic valve atresia which was subsequently confirmed at autopsy Echoes from an aortic leaflet were not obtained in these 3 patients. In 2 neonates not included in this material, narrow aortic roots were found at echocardiographic examination, but *with* an echo from an aortic cusp. At autopsy it was found that these 3 patients had a *hypoplastic left heart syndrome* with an aortic valvular stenosis. Experience of echocardiography in the diagnosis of aortic valve atresia has been briefly reported earlier (26). A small aortic root was, however only identified in 2 out of 8 patients in that material. Echocardiographic examination of the aortic root and of the mitral valve region (23) seems to be very useful in the investigation of patients with the malformations commonly referred to as the hypoplastic left heart syndrome

A comparison between echocardiographic and angiocardiographic examinations of the aortic root could be performed in 89% of the patients in the present material. The largest group of patients (9%) in whom this comparison was not made had an abnormal position of the aorta or a truncus arteriosus communis. A discussion about this group seems motivated.

This group contained *three patients* with truncus arteriosus communis. In all these 3 patients two parallel echoes similar to the aortic root echoes were obtained with the transducer in the second left intercostal space angulated in the medial-cranial direction In a normal echocardiogram from an aortic root there is an echo-free space anterior to the echo from the anterior wall of the aortic root (Fig. 3). By the contrast-echo method Gramiak et al. (12) have shown that this echo-free space is *the outflow tract of the right ventricle. In the* 3 patients with truncus arteriosus this echo-free space was replaced by dense echoes presumably originating from anterior mediastinal structures. This observation which does not seem to have been reported earlier can presumably contribute to the diagnosis of truncus arteriosus communis.

One patient with a *double outlet right ventricle* was examined. In this patient echoes presumably originating from the aortic root were obtained *far more anteriorly* than usual. It was also shown that the posterior wall of the aortic root was not at the same distance from the anterior chest wall as the echo from the anterior mitral leaflet. This observation is in agreement with that by Chesler et al. (4) of echocardiographic recognition of mitral-semilunar valve discontinuity in patients having both vessels *originating from the right ventricle*.

The largest group of patients excluded due to malposition of the aorta was 8 patients with transposition of the great arteries (dextro-transposition) *In* 6 of these patients no reliable echoes could be obtained from the aortic root or the pulmonary artery In 2 patients parallel echoes similar to those *from an aortic* root were seen with the transducer in the third *left intercostal space* at the sternal border with the transducer angulated slightly in the *lateral* direction. It can be assumed that these echoes came from the walls of the *pulmonary* artery



Fig 5 Angiocardiogram of the left atrium in lateral projection The site where the measurements of the left atrial diameter was made is indicated.

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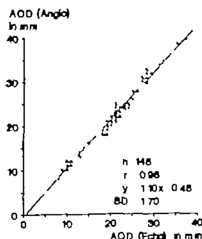


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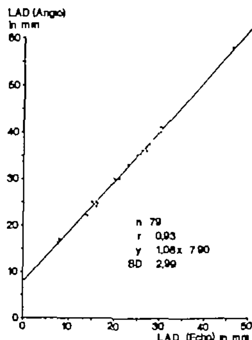


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venous return have been investigated with echocardiography They were not included in the material presented here because satisfactory estimations of left atrial size by angiocardiography could not be obtained. Three of these patients showed a left atrial dimension estimated by echocardiography below the lower normal limit and one at the lower normal limit compared with values obtained from infants without heart disease (24) These findings agree with those obtained earlier at angiocardiography (6) and at echocardiography (9)

The left atrial dimension obtained at echocardiography has been used to estimate the actual volume of the left atrium (17 18) This type of estimation is based on the assumption that the shape of the left atrium is always approximately the same and the relation between the diameters in the three directions is about the same. The validity of this estimation in the majority of cases is revealed by the good correlation between the volume of the left atrium measured on the angiocardiogram and the cube of the left atrial dimension measured at echocardiography reported by Hirata et al. (17). Exceptions do however exist, and in, for example total anomalous pulmonary venous return it has been shown that the shape of the left atrium is abnormal (6). The present investigation was not designed to compare left atrial volumes but only a left atrial dimension. The echocardiographic measurements, as used in the present investigation, can, however be regarded as a useful semi-quantitative method. It can provide additional information in the investigation of patients prior to heart catheterization and angiocardiography and it is an easily performed bedside examination in the follow-up of a patient.

Limitations to the echocardiographic estimation of aortic root diameter and left atrial size

A satisfactory recording of an echocardiogram from the aortic root left atrium could be obtained in 89% of the patients in whom a comparison of aortic root diameter was made, and in 87% of the patients in whom a comparison of a left atrial dimension was made. The main limiting factor in these examinations was the prerequisite of a normally located aortic root both for the measurement of the aortic root diameter and for the measurement of a left atrial dimension. This prerequisite could not be fulfilled in 9% of the patients in whom a measurement of the aortic root diameter was attempted and in 10% of the patients in whom a measurement of a left atrial dimension was attempted. In the remaining 2–3% no satisfactory echocardiographic recording could be obtained due to the interposition of lung tissue in front of the heart or malposition of the heart. Another factor which is an obstacle to echocardiographic examinations is deformity of the thoracic cage, but this was not met in the present material. Some infants and children were unwilling to cooperate in this examination but they could be examined after slight sedation.

SUMMARY

During a period of 18 months, a comparison was made between echocardiography and angiocardiography in every patient submitted to heart catheterization and angiocardiography This paper deals with comparisons between an aortic root dimension measured on an echocardiogram and the aortic root diameter measured on the angiocardiogram, and between a left atrial dimension measured on an echocardiogram and the sagittal left atrial diameter measured on the angiocardiogram. This study could be made in 89% of the 166 patients with respect to the aortic root and in 87% of the 91 patients with respect to the left atrial dimension. The failure to obtain measurements in some of the patients (9 and 10% respectively for the two comparisons) was generally due to an abnormal position of the aorta (transposition of the great arteries, double outlet right ventricle or truncus arteriosus communis) while in a few remaining cases no satisfactory echocardiogram could be obtained.

This observation is in agreement with the findings in a recent report about echocardiographic diagnosis of transposition of the great vessels (15) Echocardiographic identification of the *outflow vessels in transposition of the great* arteries was accomplished in all 11 patients reported by Gramiak et al (15) It was not at all possible to identify these echoes to the same extent in the present material. This discrepancy may be explained by differences in technique Another report by Gramiak et al (14) indicates that they may have used a focused transducer for this examination This type of *focused transducer* which improves the resolution in the near field (14) was not used in the present study

Finally no echoes from the aortic root were obtained in three *patients with a corrected* transposition of the great arteries (levo-transposition)

Thus the findings in the present investigation seem to allow the following conclusions. (1) If an echocardiogram as shown in Fig 3 is obtained with the transducer in the third or second left intercostal space at the left sternal border and angulated in the medial or the medial-cranial direction it is justified to assume that *this represents a normally positioned* aortic root, and (2) the aortic root diameter measured on the echocardiogram will show good agreement with that measured on the angiocardiogram

Examination of the left atrium

Hirata et al (17) have compared echocardiography and angiocardiography for the estimation of left atrial size in adults They compared the left atrial dimension on echocardiography with a left atrial area on angiocardiography and found a correlation coefficient of 0 91 Hirata et al (18) have also carried out a similar investigation to that reported here comparing left atrial dimension on echocardiography and left atrial dimension on angiocardiography in infants and *children They found* a good correlation ($r = 0.91$) in infants but a less satisfactory correlation ($r = 0.70$) in children more than 1 year of age. In the present material the corresponding correlation is fairly good ($r = 0.93$) irrespective of the age of the patient. The only difference in technique seems to be that in the present investigation it was stated as a prerequisite that an echo from an aortic leaflet must be obtained In this way the transducer position was defined both through the *location* on the precordium where the transducer should be placed and the *direction* in which an echo from an aortic leaflet could be obtained between the echoes from the aortic wall It is possible that this has ensured a better reproducibility of the measurements and can explain the better correlation seen in this material especially in children more than 1 year of age

Previously two methods have been used for the non-invasive estimation of left atrial size plain chest roentgenography and electrocardiography Hirata et al (17) made a comparison between the estimation of left atrial size by plain chest roentgenogram and by angiocardiography They found that the left atrial size could be both overestimated and underestimated by the plain chest roentgenogram Levin et al. (21) made a thorough com*parison between the various methods* for estimating left atrial size They found that the the left atrial volume had to be enlarged 2.5 times above the normal limits before plain chest roentgenogram and electrocardiogram consistently defined left atrial enlargement. Arvidsson (1) has pointed out that the left atrium has its largest volume during a very short period of time at the end of ventricular systole He concluded that there is a considerable likelihood that the left atrium appears too small at routine chest roentgenography"

Several authors have described the value of estimating left atrial size in the evaluation of patients with various forms of congenital heart disease i.e left to-right shunts distal to the atrioventricular valves (1 3 19 20 27) mitral regurgitation (10) and total anomalous pulmonary venous *return (6) It can be noted that* 4 infants with total anomalous pulmonary

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A very good correlation ($r = 0.98$) was found between the aortic root dimension as measured by echocardiography and the aortic root diameter as measured by angiocardiography. A good correlation ($r = 0.93$) was also found between the left atrial dimension as measured by echocardiography and the left atrial sagittal diameter as measured by angiocardiography.

Examination of the outflow vessel in truncus arteriosus communis by echocardiography gave echocardiograms which could contribute to the diagnosis of truncus arteriosus communis.

The conclusion of this investigation is that the echocardiographic examination as described here can serve as a useful non invasive semi-quantitative method for the estimation of the aortic root diameter and the left atrial size.

ACKNOWLEDGEMENTS

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Fig. 2. Sections of normal kidneys in a 3-year-old child. *Above*: transverse section, *below*: longitudinal sections, prone position.

Fig. 3. The kidneys under fasting condition in an 8-year-old boy. The pelves are not clearly seen. *Above*: transverse section, *below*: longitudinal sections, prone position.

Fig. 4. Scanning of the kidneys of the same child as in Fig. 3 one hour later following the consumption of 300 ml water. Meanwhile a marked dilatation of both pelves has occurred. *Above*: transverse section, *below*: longitudinal sections, prone position.

his bed. The child was routinely examined in the prone position. Scanning in the supine position was only occasionally more informative. Both longitudinal and transverse scanning were done in several planes with intervals of ½–1 cm. Picture-size was one-third of full body size.

In older children it is possible to use a coordinate system with the transcristal plane as a reference (4). This affords the possibility of reproducing the same sections with some time interval. In smaller children, however, it is impossible to use this system because the child only remains in the same position for a very short time.

Only a few children have been sedated before the investigation. The normal fear of the unknown is soon replaced by calmness and cooperation when the children discover that the procedure is painless and without discomfort.

A special problem unknown during the examination of adults is the sensation of tickling caused by the gliding of the transducer on the skin. This causes a rather disturbing reflex movement of the child's back which blurs the scanning picture. To avoid this a special technique is useful. In adults it is possible to build up the scanning picture by several sweeps and rocking movements of the transducer while the sensitivity-settings are adjusted in order to produce the best section picture. In children, however, the optimum sensitivity-settings must be found by some preliminary trials, and then the full picture of the section recorded in one single sweep before the child makes the reflex movement of the back. It is also useful to press the transducer slightly against the skin in order to avoid the tickle. This technique together with patience and sometimes mild sedation has solved this problem.

The investigation of the kidneys and urinary tract takes 10–30 minutes depending on the age and cooperation of the child.

PATIENTS

During the period 01-02 72/01-02-73 the ultrasonic investigation of the kidneys and urinary tract was

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ULTRASOUND IN THE INVESTIGATION OF DISEASE OF THE KIDNEY AND URINARY TRACT IN CHILDREN

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During the last few years an increasing interest has developed in the use of ultrasound for the investigation of diseases of the kidney and urinary tract (2, 3 5 9 11 12)

A systematic description of the ultrasonic picture of the normal and abnormal kidney in adults has been given (1 8) but the use of ultrasound has only recently been described for the investigation of kidney diseases in children (6 10)

It is the aim of this work to demonstrate the suitability of the ultrasonic investigation in this field based on the use of this technique in a 100 children with an age range between 1 day and 15 years.

PRINCIPLES OF DIAGNOSTIC ULTRASOUND

Ultrasound at high frequencies can be transmitted through human tissues as a very narrow cylindrical bundle of pressure waves. Ultrasound is generated in a transducer where a crystal by means of electrical energy is set into oscillations of high frequency This takes place several hundred times per second with a duration of a few microseconds.

When the ultrasound wave strikes an interfase between different tissues at right angles, an echo will be reflected to the transducer which in the pauses of ultrasound generation acts as a receiver and converts the mechanical oscillations into electrical energy and this through amplification is displayed as deflections from a calibrated baseline on an oscilloscope. The calibration is based upon the known velocity of ultrasound in human tissues. This is the A mode

Ultrasonic scanning or the B-mode means that the transducer is moved in a selected plane of section on the body The position and direction of the transducer together with the received echoes are electronically transmitted to a fluorescent screen where the echoes are seen as bright dots in a sectional image of the tissues. The scanning picture remains on the screen for a certain time and can be photographed.

TECHNIQUE AND METHOD

The ultrasound apparatus used in this study was a modified Escoline 20. For the recording of scanning pictures this apparatus was used in connection with an Escoline B-scanner and a modified Tektronix 564 storage oscilloscope

The compound contact method was used with a transducer of 2,25 mHz focused on 10 cm. The diameter of the transducer was 22 mm. Olive oil was used as a coupling agent on the skin.

The investigation was carried out in the laboratory with the child lying on an examination couch or in

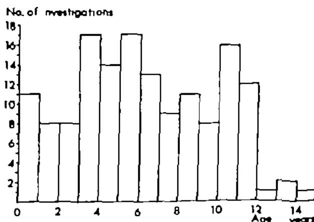
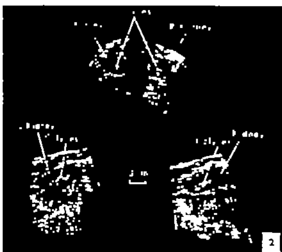
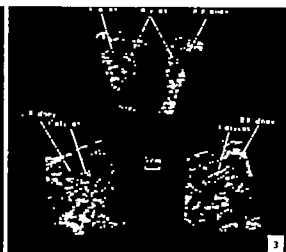


Fig 1 The age distribution of the investigated children.





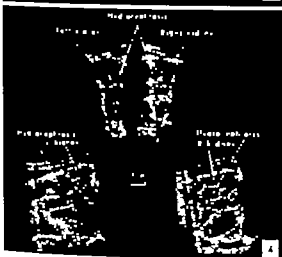


Fig. 2. Sections of normal kidneys in a 3-year-old child. *Above:* transverse section, *below:* longitudinal sections, prone position.

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The investigation of the kidneys and urinary tract takes 10–30 minutes depending on the age and cooperation of the child.

PATIENTS

During the period 01-02-72/01-02-73 the ultrasonic investigation of the kidneys and urinary tract was

carried out in 100 children with a total number of 148 investigations (Fig. 1). All the children were submitted to the paediatric department because of various symptoms from these organs, and all had an urography before or after the ultrasonic investigation. If the urography proceeded the ultrasonic examination, the result was unknown. A number of children had ultrasonic control examinations with an interval of some months.

It was possible to demonstrate and outline the position and configuration of the kidneys in all but 4 cases. These 4 cases were children in the age-group of 2–3 years where the necessary cooperation was not achieved despite sedation and several attempts.

NORMAL KIDNEY

The size In order to measure the true size of the kidney the procedure in adults is to mark on the skin the two poles of the kidney which have been found by transverse scanning in the prone position Longitudinal scanning through this axis will give the length of the kidney In small children this procedure is less useful because of the short distance between the two poles which results in an uncertain definition of the longitudinal axis. In these cases it is preferable to measure the section where the kidney looks largest.

The other measures are taken from transverse sections at right angles to the longitudinal axis. Because of the lower position of the right kidney the largest transverse sections of both kidneys are not necessarily found on the same transverse scanning

Cortex and calyces The renal cortex is acoustically homogeneous and only gives reflections at rather high sensitivity The calyceal system is seen in longitudinal scans as a central located collection of dense echoes. On transverse sections the calyceal echoes are seen located medially near the hilum of the kidney Fig 2 demonstrates the ultrasonic appearance of normal kidneys in a 3-year-old child.

Pelvis Under normal conditions the walls of the renal pelvis cannot be shown to be separated

Sometimes it has been possible to demonstrate the pelvis in kidneys after water loading Fig 3 shows the kidneys of an 8-year-old

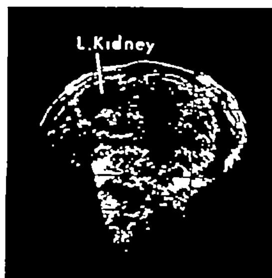


Fig 5 Transverse section in the prone position of a 3-year-old child demonstrating a slightly enlarged kidney on the left side and absence of the kidney on the right side.

boy after 12 hours without fluid The pelves are not clearly seen. Fig 4 shows the same sections 1 hour later following the consumption of 300 ml water Meanwhile a marked dilatation of the pelves has occurred. The urography was normal under ordinary fasting conditions. A miction-cysto-urethrography revealed a bilateral ureteral reflux with a retrograde filling of both pelves.

This ultrasonic demonstration of a change in the pelvic size may indicate that a more physiological impression of the renal pelvis is obtained if the kidney pelvis system is filled up before the investigation is carried out.

DISEASES OF THE KIDNEY

The ultrasonic investigation demonstrates changes in the anatomy of the kidney and urinary tract caused by congenital anomalies and various diseases. The method has no place in the demonstration of infections which in children rarely produces morphological changes in these organs.

Congenital anomalies

Renal agenesis. Unilateral non function at urography can be due to a non functioning kidney or renal agenesis. Fig. 5 demonstrates

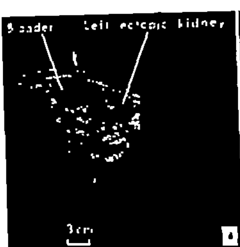


Fig. 6. Ectopic kidney in close relation to a distended urinary bladder in a 7-year-old child. Transverse section, supine position.

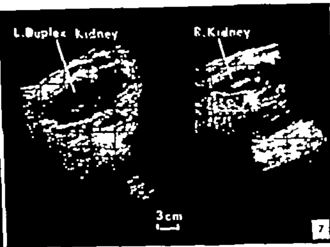


Fig. 7. Duplex kidney on the left side in a 3-year old child. Longitudinal sections, prone position.

in a 3-year-old boy a slightly enlarged kidney on the left side with absence of the right kidney.

Ectopia renis. If a kidney is missing on its normal place an ectopic kidney in the abdomen or pelvis must be ruled out. In order to find a kidney behind the bowel with its content of total reflecting gas it is necessary to press slightly on the abdominal wall with the hand to bring the gas away from the search area. If the kidney is situated close to the abdominal wall or to a distended bladder it is easily located (Fig. 6).

Ren duplex. The kidney is often larger than normal and larger than the other. It is possible to demonstrate the calyceal echoes divided into two major groups (Fig. 7).

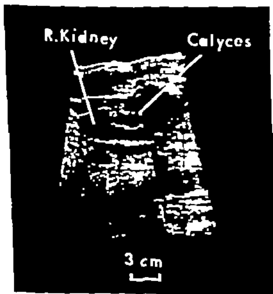


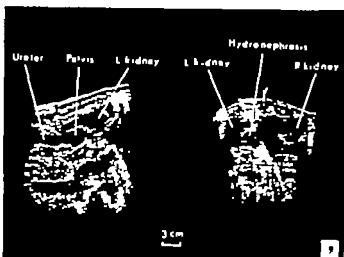
Fig. 8. Cystic ovoid structure in the pelvic region in a 12-year-old girl with a small hydronephrosis. Longitudinal section, prone position.

Space-occupying lesions

Tumor renis. Although tumours of the kidney in childhood are rare the ultrasonic investigation is a valuable method in the demonstration of a kidney tumor using the same criteria as in adults (1, 8).

In answer to the question whether a tumour is solid or cystic it is necessary to study the A-mode because of its intensity modulation. This means that all echoes regardless of their strength will be registrated, as opposed to the B-mode where an "on-off" system removes reflections below a certain limit of strength.

Hydronephrosis. The ultrasonic picture of this condition depends upon the size of the pelvic system. An early sign is a splitting-up of the calyceal echoes or the appearance of a cystic structure of ovoid shape in the pelvic area (Fig. 8).



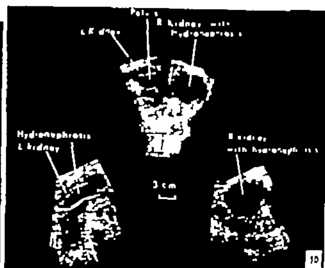


Fig 9 Hydronephrosis and hydro-ureter in a 5-year old girl. *Left* longitudinal section *right* transverse section prone position.

Fig 10 Congenital hydronephrosis in a 2 year-old boy The scanning shows no visible cortex on the right side On the left side a narrow cortex zone is seen together with a marked dilatation of the pelvis. *Above* transverse section *below* longitudinal sections, prone position

In more severe cases the distended pelvis can clearly be demonstrated in both longitudinal and transverse scanning. Occasionally it is possible to follow a dilatated ureter on its way down to the iliac crest (Fig. 9)

The terminal phase of a congenital hydronephrosis in a 2 year-old boy is seen in Fig. 10 On the right side there is no visible cortex. On the left side only a very narrow cortex zone remains and a marked dilatation of the pelvis has occurred.

The advantage of the ultrasonic investigation in hydronephrosis is obvious because of the possibility of following changes in the condition without risk or discomfort to the child Furthermore it is possible to demonstrate a hydronephrosis in a non-functioning kidney

Cysts A cyst is normally well demonstrated because of the absence of echoes even at a high sensitivity setting Another characteristic is the very well-defined back wall. The differentiation between a cyst and a hydronephrosis is presumably only possible if there is a visible structure of an otherwise normal kidney without disarrangement of the calyceal echoes.

The supra-renal gland The investigation of the kidneys includes an examination of the suprarenal gland because of the close anatomical relation It has not been possible to demonstrate the gland under normal conditions. This is probably because of its small size and its unapproachable position. If it is difficult to demarcate the upper pole of the kidney from the liver or the spleen it may give the impression of an enlarged suprarenal gland.

The ultrasonic picture of a tumor in the right suprarenal gland in a 1-day-old baby is seen in Fig. 11 The operation revealed a haematoma inside the gland.

ULTRASONICALLY GUIDED PERCUTANEOUS PUNCTURE OF THE KIDNEY

Ultrasonic investigation before and during the performance of a biopsy of the kidney demonstrates the position and configuration of the kidney The direction and distance to the area of biopsy is visualized.

When a specially constructed ultrasonic transducer is used with a central canal through which the puncture needle is inserted the needle will follow the direction of

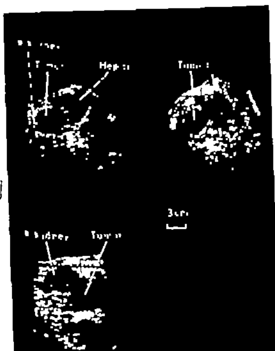


Fig. 11. Tumour of the right suprarenal gland in a 1-day-old child. *Upper left:* longitudinal section in the supine position. The tumour is located between the liver and the kidney. *Upper right:* the tumour in a transverse plane in the supine position. *Below:* longitudinal section in the prone position demonstrating the tumour at the upper pole of the kidney. The lobulation of the infantile kidney persists. The operation revealed a haematoma inside the suprarenal gland.

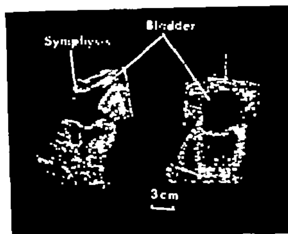


Fig. 12. The urinary bladder in a 2-year-old child. Estimated volume 50 ml. *Left:* longitudinal section, *right:* transverse section, supine position.

the ultrasonic beam (7). With this equipment it is a very precise method which can be used in biopsy of the cortex. The same technique is valuable in aspiration of urine from both pelves in order to estimate a difference in bacteriology or functioning of the two kidneys. In this way it is also possible to produce direct pyelograms when normal catheterisation of the ureter is impracticable.

DEMONSTRATION OF RESIDUAL URINE

Demonstration of the presence of residual urine (Fig. 12) by ultrasonic examination saves the child from an unpleasant and sometimes painful catheterisation with its risk of infection. In most cases it is sufficient to get a rough impression of the amount of the residual urine but it is also possible to determine the outer shape of the bladder and then by means of the proper formula to calculate the amount approximately.

CONCLUSION AND SUMMARY

Based upon the use of the ultrasonic investigation of diseases of the kidney and urinary tract in 100 children it can be concluded that it is a very useful method in the demonstration of the anatomical changes that occur in these organs during certain diseases.

The reliability of this new diagnostic method awaits further evaluation, but so far the ultrasonic investigation has been found to be a valuable complement to radiology in the primary diagnostic approach, as well as in the supervision of the subsequent changes in kidney and urinary tract diseases in children.

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INFANT MORTALITY

Causes of Death During the First Year of Life in a Five-year Series

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It is well known that neonatal mortality rates have not fallen to the same extent as mortality during the rest of infancy. It was therefore considered worth while to analyse the causes of neonatal mortality today in order to obtain a better understanding of the causal factors. This paper concerns an analysis of the causes of death within the first year of life in a 5-year material in a well defined geographical area (town of Malmö), where the population as well as the principles of medical treatment have been relatively stable during this period. Interest was concentrated mainly on neonatal mortality in infants with low birthweight. The limits of the neonatal period and low birthweight are generally accepted, but nevertheless arbitrary. To obtain a more complete picture we therefore included foetal deaths as well as deaths within the first year of life in all weight groups.

DEFINITIONS

Low birth weight (LBW) is to be understood as a birthweight of at most 2 500 g.

Perinatal mortality includes stillbirths after the end of the 28th week of pregnancy and liveborns who survived for at most 6 days, calculated relative to all births.

Early neonatal mortality includes all liveborn infants, who died before the seventh day of life calculated relative to liveborns.

The term *liveborn* designates all infants who showed signs of life, such as respiration and/or cardiac activity irrespective of the duration of pregnancy and birthweight.

The above definitions are those recommended by WHO and are used in Swedish vital statistics.

The duration of pregnancy was calculated from the first day of the last menstrual period. Infants born before the end of the 38th week of pregnancy (i.e. 266 days or less) were designated pre-term, in accordance with Swedish recommendations valid at the beginning of this investigation (8). Infants born in the 39th to 42nd week of pregnancy were classified as delivered at *term* and those born from 43rd week inclusive as *post-term*.

The infants were classified as small for gestational age (SGA) when they had a birthweight below the 10th percentile according to Swedish standard curves (7), appropriate for gestational age (AGA) between the 10th and 90th percentile and large for gestational age (LGA) when above the 90th percentile.

Since the lower limit of this standard is the 33rd week of pregnancy, infants born after a shorter pregnancy were classified according to Usher & McLean's standard curves (16).

The term premature was avoided because its definition varies, though it is, as a rule, used synonymously with LBW according to WHO.

MATERIAL AND METHODS

The material was collected in Malmö in 1966–70. The town has a population of about 260 000 inhabitants. The socio-economic standard of the population is, on the average, good and has not changed substantially during the 5-year period in question. The town has only one hospital for somatic diseases, where all de

Table 1 *All births in the town of Malmo 1966–70*
Perinatal neonatal and infant mortality

Year	Liveborn	Stillborn >28 weeks	Dead within 6 days	Perinatal mortality (‰)	Neonatal mortality ( )	Dead 1 week–1 year	1 st year mortality (%)
1966	3 841	43	34	1 98	0 89	17	1.33
1967	3 736	39	35	1 96	0 94	19	1 45
1968	3 516	22	26	1 36	0 74	6	0 91
1969	3 298	19	39	1 75	1 18	9	1 46
1970	3 414	18	38	1 63	1 11	8	1.35
Total	17 805	141	172	1 74	0 97	59	1.30

liveries take place and only one children s hospital where all sick children are cared for Treatment of the mothers and of the children was therefore uniform During the 5-year period, meetings of the obstetricians, pediatricians and pathologists were regularly held and all cases of perinatal diseases and death were thus analysed while they were of current interest.

Information on the total number of deaths within the first year of life was received from the parish offices in the town Supplementary information was obtained from the hospital records of the mothers at the maternity department and the children s records at the children s hospital or where else they had been cared for The number of stillborn and liveborn children and those who died in infancy is given in Table 1

The frequency of LBW is given in Table 2. The infants with LBW were divided into 4 groups according to the duration of pregnancy The number of infants born alive in each group and the number that died in the first year are given in Fig. 1 The infants *are grouped according to birthweight in Fig 2. In* each 500 gram group they are divided in SGA and AGA None of the LBW infants was LGA

Of the 231 children who died in infancy 143 were boys and 88 were girls, i e a male–female ratio of 1.55 1 The preponderance of males in infant mortality is well known (12)

All infants were examined *post mortem* except 2 whose parents would not give their consent. In these 2 cases the clinical diagnosis was taken as the cause of death. 217 were examined at the Department of Pathology in Malmö and 12 at other institutions, from which data were obtained. The necropsies included examination of the internal organs and the brain. Material from the lungs, kidneys, myocardium, thymus and adrenals were examined microscopically All 217 lung specimens from cases necropsied in Malmö were re-examined and the cases were grouped according to the presence or absence of hyaline membranes, haemorrhages and inflammations. This re examination included all LBW infants except 2 who died from gross malformation.

Each case was classified according to one pathological-anatomical diagnosis, though such a classification must necessarily be arbitrary since several factors may have contributed to the fatal issue. The following diagnostic groups were used.

Malformations comprising severe malformations of the heart central nervous system gastro-intestinal tract and urogenital system as well as multiple grave *external and internal malformations that* could explain the death

Haemorrhages comprising three different types.

1 Intraventricular haemorrhages in the brain. These cases were further classified according to the presence or absence of hyaline membranes in the lungs.

2. Massive mainly intra-alveolar lung haemorrhages.

3 Two cases of haemorrhagic destruction of the

Table 2. *LBW infants born in Malmö 1966–70*
Neonatal and infant mortality

Year	Liveborn	Birthweight (<2 500 g)	LBW (%)	LBW dead within 6 days	Neonatal mortality in LBW (%)	LBW dead 1 week–1 year	1st year mortality LBW (%)
1966	3 841	188	4 9	26	13 8	6	17.0
1967	3 736	203	5 4	25	12.3	4	14.3
1968	3 516	192	5.8	19	9 9	2	10.9
1969	3 298	198	6.3	29	14 7	4	16.7
1970	3 414	198	6 1	30	16.0	1	15 7
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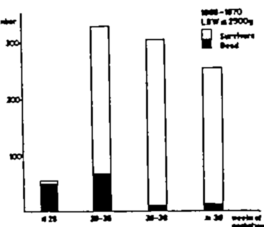


Fig. 1. All LBW-infants during the 5-year period grouped after gestational age and infant death in each group.

suprarenals and one of haemorrhagic destruction of the pons and cerebellum.

Hyaline membranes were graded as mild (+) with scattered membranes in single alveolar ducts, moderate (++) with fairly widespread presence of hyaline material lining the alveoli in different parts of the lungs and severe (+++) with widespread, coalescent, eosinophilic linings in larger areas of the alveolar ducts and alveoli.

Immaturity was used to designate poorly aerated and immature lungs in low-weight infants of short gestational age where no other morphological explanation of death could be found. Those cases could also have been classified as asphyxia, which was the clinical diagnosis. This diagnosis was, however, used only in a few cases. These infants had grave post partum asphyxia, from which they never recovered, no morphological findings and more mature organs. Together with all the remaining causes of death they were assigned to a miscellaneous group.

TREATMENT OF THE INFANTS

Treatment of the infants influences the duration of survival as well as the definitive prognosis and is therefore briefly described below.

Resuscitation of asphyxiated infants was carried out at the delivery ward. The principles of resuscitation were: immediate care of the infant with clearing of the upper airways, administration of oxygen by mask and bag or by oral endotracheal tube, and when necessary correction of acidosis and external cardiac massage. Measures were also taken to reduce loss of heat. 7 infants were transferred directly to the surgical or neurosurgical department for operation because of malformations, 5 were transferred to a special ward for treatment with an intermittent positive pressure ventilator. In 14 cases primary resuscitation was unsuccessful. All the other infants who died in the neonatal period were being cared for in the neonatal ward at the children's hospital at the time of death. Of these, 121 infants were LBW. Treatment consisted of incubator care with continuous supervision and administration of oxygen. Oral feeding consisted of glucose and breastmilk, mostly given by naso-gastric tube. In 69 cases glucose infusion was given via catheter in the umbilical vein and in 30 cases in an umbilical artery catheter. The use of umbilical artery catheter was introduced in 1969. Sodium bicarbonate was given for acidosis correction. Exchange transfusion was done in 5 cases. Antibiotics were used when bacterial infection was suspected or verified. Ampicillin was also used prophylactically when umbilical vein catheters were inserted in 1966–67.

RESULTS

The distribution of the morphological diagnoses among the 231 infants who died within the first year of life is given in Table 3.

Table 4 gives the age distribution of the mothers compared with a normal material from the same area and the same period.

The obstetric histories of the mothers and the complications of pregnancy and delivery are given in Table 5. The frequency of toxicosis of pregnancy was 8.6% in our material compared with 9.2% in all births. In 8 of the 10 infants who died from hyaline membrane

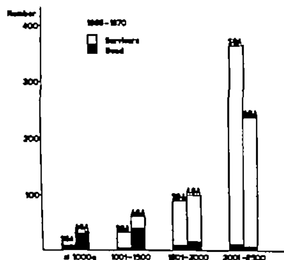


Fig. 2. All LBW-infants during the 5-year period in 500 gram groups and death within one year in each group.

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1967	3 736	39	35	1 96	0 94	19	1 45
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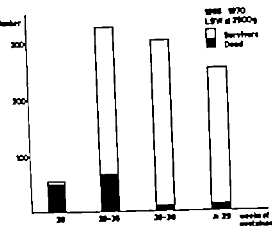


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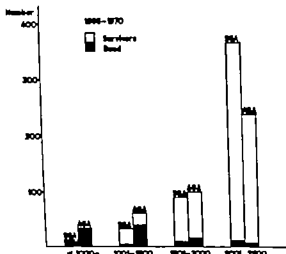


Fig. 2. All LBW-infants during the 5-year period in 500 gram groups and death within one year in each group.

Table 3 *The main cause of death in 231 infants dead within first year of life*
LBW in 500 gram birthweight groups

Diagnosis	Birthweight (grams) <1 000	1 001–1 500	1 501–2 000	2 001–2 500	>2 500	Total
Malformations	1	3	6	8	47	65
Cardiovascular	—	1	1	1	24	27
Cerebrospinal	—	—	—	2	10	12
Gastrointestinal	—	2	1	2	9	14
Urogenital	1	—	1	2	—	4
Multiple	—	—	3	1	4	8
Immaturity	29	7	2	—	—	38
Haemorrhage	13	10	2	1	5	31
Intraventricular	9	1	—	—	—	10
Intraventr + hyal. membr	3	3	—	1	—	7
Pulmonary	1	6	2	—	2	11
Other	—	—	—	—	3	3
Hyaline membranes	7	21	13	10	10	61
+	4	9	3	1	3	20
+ +	3	9	7	6	5	30
+ + +	—	3	3	3	2	11
Miscellaneous	1	4	4	4	23	36
Asphyxia	—	—	—	2	2*	4
Erythroblastosis	—	1	1	—	2	4
Inflammation neonatal	1	2	—	1	3	7
Inflammation >1 week	—	1	1	—	8	10
"Sudden unexpected death"	—	—	1	1	5	7
Accident	—	—	1	—	3	4
Total	51	45	27	23	85	231

* Necropsy not allowed by parents in one case.

disease and who had a birthweight of more than 2 500 g the mothers had had serious complications of pregnancy such as diabetes, severe toxicosis of pregnancy Rh immunisation and bleeding This frequency of complications of pregnancy was even higher than among the mothers of LBW infants with hyaline membrane disease where it was about 50% 18 twins died during the neonatal period. They constituted 10% of all infants who died neonatally while the incidence of twins in the entire material was 0.86% 19 twins were born alive and died within the first year of life. They belonged to 15 pairs. In 4 cases both twins died in 3 the other twin had been born dead, and in the remaining 8 the other twin had survived. 10 of the twins were born before the end of the 28th week, only 2 after the 34th week. All of them were LBW and all except 1 died within the first week 8 were firstborn and of those 3 died from hyaline membrane disease, compared with 7 of the 11 second born The material did not include any triplets. All of 4 quadruplets with a birthweight of 560 to 1 090 g and born in the 28th week of pregnancy after hormone therapy of the mother died on the first day 3 of them had hyaline membrane disease, the fourth was only immature

Table 4 *Maternal age in a normal material LBW and malformed infants who died during the first year of life*
The normal material consisted of all normal births during a twelve month period 1966–67

Maternal age (years)	Normal material n	%	Low birthweight n	%	Malformations n	%
<19	346	11	18	13	5	8
20–24	1 165	36	58	41	16	25
25–29	1 082	33	44	31	25	• 39
>30	621	20	23	16	18	28
	3 214	100	143	100	64	100

Table 5 *Outcome of former pregnancies and complications in pregnancy in question in mothers with infant deaths*

	LBW no malformations		All malformations		Birthweight 2 500 g no malformations		All births 1966–70
	n		*n*	%	*n*	%	
Mothers	120		65		39		
Primigravidae	47	39.2	31	47.7	14	36.8	About 50 *
Former deliveries without complications	33	27.5	28	43.1	16	42.1	
Former pregnancy complications	40	33.3	6	9.2	9	23.7	
Number of healthy children	48	(0.7/mother)	46	(1.4/mother)	[illegible]	(1.0/mother)	
Diabetes	2	1.7	4	6.1	4	10.5	0.29
Placenta praevia	6	5.0	—	—	—	—	0.23
Ablatio placentae	8	6.7	1	1.5	6	15.8	0.56
Urinary tract infection (incl. bacteriuria)	4	3.3	5	7.7	5	13.[illegible]	3–4 *
Breech delivery	23	19.2	9	13.8	3	7.9	3.1 *
Caesarean section	18	15.0	4	6.1	6	15.8	3.1 *

The exact number not known, because bacteriuria treated during pregnancy is not always registered.

The duration of survival of the infants was studied for any correlation with birthweight (Fig. 3), or with diagnosis (Fig. 4). The mean birthweight and mean duration of *pregnancy* was studied for the three main causes of death, malformations, hyaline membrane disease and immaturity (Table 6).

The frequency of a low Apgar score at 1 min (≤6 points) was 40% in the group with hyaline membranes and 63% in the immature group without other morphological findings.

The frequency of gross malformations found in stillborns and infants who died are presented in Table 7.

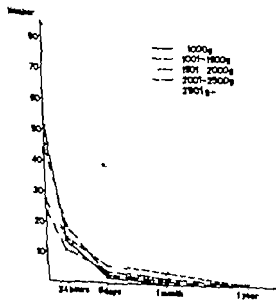


Fig. 3 Duration of survival in different weight groups.

DISCUSSION

Though perinatal mortality rates have been reduced in recent decades, it is still 2–4% also in *countries* with a *fairly* high standard of living and a well organised medical service. Several large investigations have been reported in Great Britain and the USA (4, 15), as well

Table 6 *Mean birthweight and mean gestational age in the 3 main diagnostic groups*

	Immaturity	Hyaline membranes	Malformations
Mean birthweight (g)	909	1 877	2 968
Range	330–1 700	570–4 430	930–4 540
Mean gestational age (days)	188	222	266
Range	161–233	173–289	172–301
Small for gestational age (%)	11	18	23

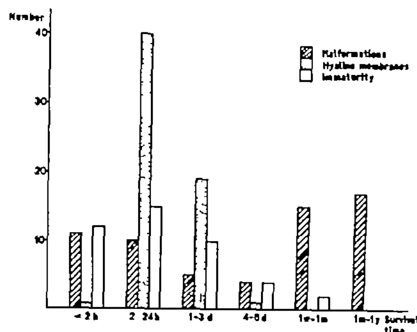


Fig 4 Duration of survival in the three main diagnostic groups, malformations, hyaline membranes and immaturity

as in the Scandinavian countries (2, 6 11) The main causes of neonatal death are malformations, asphyxia hyaline membranes and intracranial haemorrhages and there is general agreement that pre term delivery and LBW are predisposing factors, (3 9 18) The causes of congenital malformations, foetal deaths and LBW must to a large extent be sought in the mother

Clifford (5) underlines the increased risk of infant mortality among very young mothers, mothers with diseases, those of low socio-economic status and grand multiparity He feels that good maternal care should be able to reduce the frequency of LBW to 6–8% and the perinatal mortality to less than 3% These results are well achieved in our series (Table 1 2) Our material is representative compared with Swedish official statistics (24 25) Neonatal mortality in Sweden 1966–70 was 0 88% and perinatal mortality 1 77%

In recent years it has often been stressed that one should consider not only low birth weight but also the duration of pregnancy and that children born after a short period of gestation are thus less mature and have poorer prospects of survival (21)

It is well recognized that necropsy is important to ascertain the cause of death since the clinical picture is often difficult to interpret and can hardly distinguish between different possible causes of death such as intracranial haemorrhage pulmonary complications and immaturity (1 3 10 17 19 22).

Stillborns

The causes of foetal deaths were not analysed except concerning malformations (Table 7). It is often impossible to give a clear-cut diagnosis even at necropsy because of maceration of the foetus. The number of stillborns was nevertheless included in the analysis in order to obtain complete figures for the perinatal mortality and to illustrate the standard of the obstetric care

The decrease in the number of stillborns during the period seen in Table 1 was tested with the chi-square method and found to be statistically significant ($p<0\ 01$) There was

Table 7 *The frequency of gross malformation in stillborns and infants who died within one year*

	Total	Malformations	%
All births	17 946	90	0.50
Stillborns	141	25	17 7
Infant deaths	231	65	28.0

on the other hand no demonstrable reduction in the total perinatal or infant mortality. Basic factors in late foetal and early neonatal death are often the same. One might therefore imagine that the decrease in the number of foetal deaths would only raise the number of births of liveborns with extremely poor prospects of surviving the neonatal period. But this was less likely because the number of infants born after extremely short gestation and with very low birthweight did not increase during the latter part of the 5-year period. The number of infants with malformations, both stillborns and infants who died within the first year of life, also remained largely unchanged.

Malformations

65 infants (0.37%) including 18 with LBW died from severe malformations. This agrees closely with Swedish official statistics (24) where malformation was given as cause of death in 0.32% during 1969–70. If the stillborns be included, the frequency of congenital malformations with a fatal issue within the first year of life will be 0.5%. Malformations of the heart were predominant (Table 3). Some of the infants with the most severe malformations died early but many survived longer and, between 7 days and 1 year the congenital malformations were the predominant cause of death.

The mothers of malformed children seemed to be older than those in a normal material from the same period. The differences between the percentages were rather small and, as the number of mothers of malformed children was small too, it could not be proved that those differences were not due to random variations. We applied a chi-square test, more precisely a test of homogeneity at a 5% level (Table 4). Only a few had diseases known to predispose to malformation of the infant, e.g. diabetes (Table 5).

Haemorrhages

Intracranial haemorrhages were found to be the cause of death in 17 cases (7.4% of total infant mortality 13.2% of neonatal mortality in LBW infants). These were all massive, intraventricular haemorrhages. Smaller subdural haemorrhages were about equally common but they were not in any case thought to be the main cause of death. As the brains were not studied microscopically other types of hypoxic lesions could not be evaluated in this material.

Intraventricular haemorrhages were found exclusively in infants with LBW 12 of them had a birthweight of 1 000 g or less. In 7 of the infants the intraventricular haemorrhage was combined with hyaline membranes. Compared with other necropsy series, the frequency of intracranial haemorrhage was low. Washington et al. (18, 19) reported 45.6% severe intracranial haemorrhages in infants that weighed less than 2 268 g. Unlike other authors (10), they found no increase in the frequency of intracranial haemorrhages in infants with hyaline membranes. In the present investigation the frequency of intracranial haemorrhages in infants with hyaline membranes was 12.3% i.e. roughly the same as in infants where no hyaline membranes were found at necropsy where it was 11.3% (12.2% if malformations are excluded).

Hyaline membranes

This was found to be the main cause of death at necropsy in more than one-third of the neonatal deaths, and thus was equally common as death from malformation. Hyaline membranes were also found to a minor extent in cases with pneumonia, pulmonary haemorrhage and cerebral haemorrhage, and thus were seen in all together about 50% of LBW infants. For comparison it may be mentioned that Ahvenainen (1) reported hyaline membranes in 25% of the cases. This suggests that other causes of death had been reduced in our material, and that hyaline membranes were therefore a more common finding. In a more recent study by Valdes-Dapena & Arey 1970 (17) the frequency of neonatal deaths from hyaline membranes was also relatively high, viz. 35%.

Hyaline membrane disease is still an enigmatic condition. The clinical picture is more often described as respiratory distress syndrome and a good review of the newer aspects of this syndrome is given by Scopes (14) among others. It occurs mainly but not exclusively in pre term infants most of whom are appropriate in weight for gestational age, and this was the case also in our material. It was not the most pre term infants who had hyaline membranes, however but they were on average heavier and had a somewhat longer period of gestation than those infants who *died of immaturity without other morphological findings* (Table 6) The difference in mean birthweight and the difference in mean gestational age in these two groups were statistically analysed and each was found to be due to random variations with a probability less than 0 1% ($p<0\ 001$) We used the ordinary estimates of means and standard deviations and the tests were based on the fact that an arithmetic mean of identically distributed random variables is approximately normally distributed.

Infants with a higher birthweight tended to have more widespread hyaline membranes at necropsy (Table 3) Infants who survived longer also had more pronounced hyaline membranes. The same observations were made by Potter & Davis (13). The role played by asphyxia in the development of hyaline membranes has been emphasised. A low 1 minute Apgar score was, however less common among infants with hyaline membranes than among more immature infants with no obvious morphological cause of death. It was noteworthy on the other hand that the second twin had severe hyaline membranes more often than the first-born twin This phenomenon is well known and has been ascribed to asphyxia during delayed delivery of the second-born twin

Immaturity

Our material included 38 infants (0 2%) who had died from immaturity i e. in whom the main finding was general immaturity particularly of the lungs, but no other demonstrable cause of death. Clinically these infants had an anoxia with shallow irregular respiration. Many died even on the first day but one third lived for 1–6 days. The mean weight in this group was less than 1 000 g and the mean duration of gestation less than 28 weeks (Table 6) Most of the infants in this group could not thus, according to conventional criteria be regarded as viable. Infants with a birthweight of 1 000 g or less constituted one-fourth of the total neonatal mortality in the present material but only 0.3% of all the infants born. 23% of these very low weight pre term infants were members of multiple births (3 quadruplets and 9 twins)

Inflammation

This group consisted of 7 infants with neonatal pneumonia after severe birth asphyxia, and 10 who died from fulminant bacterial or viral infections later in infancy Inflammation thus was the cause of 7 4% of all infant deaths and 4 1% of neonatal deaths. Less than 0 1% of all infants born during the 5 year period died from inflammation Compared with other necropsy series, this figure is very low (1 3 17) Less pronounced inflammatory changes were found mainly in the lungs in some of the other cases but were considered as of minor importance and not contributory to death

Survival

The duration of survival of the infants was closely correlated with birthweight (Fig 3) Of the infants in the 2 lowest weight groups, i.e. 1 500 g or less, two-thirds died within the first 24 hours of life and of all LBW infants, who died during the first year 88% did so in the early neonatal period. Only 50% of the infants who had a birthweight of more than 2 500 g and who died during the first year did so within the first week.

The survival time was also correlated with the diagnosis (Fig 4). Two-thirds of the

deaths from hyaline membrane disease occurred 2 to 24 hours after birth and all but 1 within 3 days.

LBW

The highest risk of early neonatal death was found among infants with low birthweight especially if they were also pre term.

Our material, collected from a well defined geographical area, where all deliveries and all infantile deaths are recorded had a low perinatal mortality and low infant mortality (Table 1). The frequency of LBW was rather low and the maternal care was extensive. The socio-economic standard was relatively good. Division by social group did not seem to have any meaning, because the methods are too crude to distinguish the differentiations in Sweden today It has been claimed that the mortality in higher social groups has not fallen at the same rate and is thus more difficult to depress (2) The negative effect of a low socio-economic standard has also been described as being more pronounced in infants with a birthweight of 1 500 to 2 500 g than the very low-weight infants, i.e. 1 500 g and less (20). This is in good agreement with our series, where in spite of good social standard the mortality was still very high when birthweight was 1 500 g or less.

LBW-infants were overrepresented not only in the neonatal period, but also in later infancy in cases of clear-cut infection (6 of 17) and "sudden infant death" (2 of 7) (Table 3). In these cases social factors may have played a role, but are difficult to analyse in detail.

Maternal diseases

The commonest complications of pregnancy and delivery in mothers of children who died in infancy are given in Table 5 The frequency of diabetes, placenta praevia and ablatio placentae were many times higher than expected. On the other hand toxicosis of pregnancy was not more common among the mothers of infants who died, nor was urinary tract infections (incl. bacteriuria) among LBW-children. The frequency of urinary tract infections was high among mothers with infants >2 500 g. The number is, however too small for any conclusion without further analysis of the cases. The number of breech presentations was markedly increased which can be partly explained by the high frequency of LBW The high frequency of caesarean section is explained by the fact that complications of pregnancy and delivery were so common.

CONCLUDING REMARKS

This material reflects the difficulty in reducing infant mortality further During these 5 years there has been a reduction in late foetal mortality but neonatal and infant mortality have remained largely unchanged. Infant mortality today is confined mainly to the neonatal period and is due chiefly to congenital malformations, immaturity and hyaline membranes. Congenital malformations are difficult to prevent. Both hyaline membranes and death from immaturity without other morphological findings occur most often in very low-weight infants with short gestational periods.

The prognosis of these conditions can be improved to some extent by more efficient paediatric care. The causes of LBW and preterm delivery are, however to a great extent to be found in the mother (5 20) Close cooperation between the obstetrician and paediatrician is therefore essential to save more children. A question that then arises is: What are the prospects for those children with the same risk factors but who survive the neonatal period and the first year of life? Does their survival increase the frequency of children with chronic diseases or do those measures that suppress mortality also suppress morbidity? The present investigation will be followed up by an analysis of the morbidity in the risk groups in the same population.

Hyaline membrane disease is still an enigmatic condition The clinical picture is more often described as respiratory distress syndrome and a good review of the newer aspects of this syndrome is given by Scopes (14) among others. It occurs mainly but not exclusively in pre-term infants, most of whom are appropriate in weight for gestational age and this was the case also in our material. It was not the most pre term infants who had hyaline membranes however but they were on average heavier and had a somewhat longer period of gestation than those infants who died of immaturity without other morphological findings (Table 6) The difference in mean birthweight and the difference in mean gestational age in these two groups were statistically analysed and each was found to be due to random variations with a probability less than 0 1% ($p<0\,001$) We used the ordinary estimates of means and standard deviations and the tests were based on the fact that an arithmetic mean of identically distributed random variables is approximately normally distributed.

Infants with a higher birthweight tended to have more widespread hyaline membranes at necropsy (Table 3) Infants who survived longer also had more pronounced hyaline membranes. The same observations were made by Potter & Davis (13). The role played by asphyxia in the development of hyaline membranes has been emphasised. A low 1 minute Apgar score was however less common among infants with hyaline membranes than among more immature *infants with no* obvious morphological cause of death. It was noteworthy on the other hand that the second twin had severe hyaline membranes more often than the first-born twin This phenomenon is well known and has been ascribed to asphyxia during delayed delivery of the second born twin.

Immaturity

Our material included 38 infants (0 2%) who had died from immaturity i.e in whom the main *finding was general immaturity* particularly of the lungs, but no other demonstrable cause of death Clinically these infants had an anoxia with shallow irregular respiration. Many died even on the first day but one-third lived for 1–6 days. The mean weight in this group was less than 1 000 g and the mean *duration of gestation less than 28 weeks* (Table 6) Most of the infants in this group could not thus, according to conventional criteria, be regarded as viable Infants with a birthweight of 1 000 g or less constituted one-fourth of the total neonatal mortality in the present material but only 0.3% of all the infants born. 23 of these very *low weight* pre term infants were members of multiple births *(3 quadruplets and 9 twins)*

Inflammation

This group consisted of 7 infants with neonatal pneumonia after severe birth asphyxia, and 10 who died from fulminant bacterial or viral infections later in infancy Inflammation thus was the cause of 7 4% of all infant deaths and 4 1% of neonatal deaths. Less than 0 1% of all infants born during the 5 year period died from inflammation Compared with other necropsy series, this figure is very low (1 3 17) Less pronounced *inflammatory* changes were found mainly in the lungs in some of the other cases but were considered as of minor importance and not contributory to death.

Survival

The duration of survival of the infants was closely correlated with birthweight (Fig 3) Of the infants in the 2 lowest weight groups, i.e. 1 500 g or less two-thirds died within the first 24 hours of life and of all LBW infants, who died during the first year 88% did so in the early neonatal period Only 50% of the infants who had a *birthweight of more than* 2 500 g and who died during the first year did so within the first week.

The survival time was also correlated with the diagnosis (Fig 4) Two-thirds of the

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PLACENTAL TRANSFUSION IN INFANTS OF DIABETIC MOTHERS ELUCIDATED BY PLACENTAL RESIDUAL BLOOD VOLUME

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In normal infants it has been shown that the clamping technique (6 7) is of significance for the size of the placental transfusion. Early clamping deprives the child of the placental transfusion, while late clamping allows the placental transfusion to take place.

In normal infants, the method of delivery (vaginal or abdominal) influences the haemodynamic state during delivery in at least 2 ways. Cassady (4) has shown that vaginally delivered infants have less total body water than those delivered by caesarean section that is, vaginally delivered infants are less hydrated than those delivered by caesarean section. This is supported by the higher serum protein concentration found in umbilical cord blood of vaginally delivered infants (3 5). The method of delivery is also important in the size of the placental residual blood volume and, therefore, for the size of the infant's blood volume since with early clamping vaginally delivered infants have a larger placental residual blood volume than those delivered by caesarean section (11)

Kjeldsen & Pedersen (8) have shown that infants of diabetic mothers (IDM) receive a larger placental transfusion than infants of non-diabetic mothers (non-IDM), measured by the placental residual blood volume. Furthermore, it has been shown that shortly after delivery IDM reduce their plasma volume to a lower level than do non-IDM (9). These differences, taken together with clinical observations of plethora in IDM could suggest a relative hypervolaemia in IDM at delivery

In earlier studies on IDM however the method of delivery and the clamping technique have not been taken into account at the same time in the calculation of the size of the placental transfusion. Since in the neonatal period, IDM have an increased frequency of respiratory distress syndrome (RDS), which in non-IDM has been related both to the method of delivery and to the clamping technique (12, 14 15 16 17) we wished with the different placental residual blood volumes to investigate the significance of these differences for the size of the placental transfusion in IDM.

MATERIAL AND METHODS

The placental residual blood volume (PRBV) was determined using Redmond's method (13) in 58 infants of diabetic mothers, and in 65 infants of non-diabetic mothers.

The material is shown in Tables 1 and 2

At vaginal delivery early clamping of the cord is defined as clamping about 5–20 sec after birth, and as a rule before the infant's cry—i.e. clamping as early as possible. At caesarean section immediate cord clamping is maintained in the early clamping group. Late clamping is clamping after stimulation and suction of the infant—i.e. about 3 min after vaginal delivery and about 1 min after caesarean section.

For the vaginal deliveries, either N_2O-O_2 or Trichlorethylene (Trilene) was given to provide analgesia. All

SUMMARY

In the years 1966–1970 all together 17 805 children were born in Malmö (population about 260 000) 141 were stillborns and 172 infants survived at most 6 days, which means a perinatal mortality of 1 74% and neonatal mortality within 6 days of 0 97% The infant mortality was 1 3% These figures agree closely with Swedish vital statistics 5 4% were LBW infants with a neonatal mortality of 13 3 ‰ 231 infants died during the first year and necropsies were performed in all but 2 The main causes of death were malformations in 65 cases haemorrhages in 31 and hyaline membranes in 61 In 38 children with a birthweight below 1 700 g post mortem examination revealed no morphologic changes except immaturity Diseases of the mother and complications of pregnancy and delivery were more common among mothers of children with low birthweight. The causes of infant mortality in countries with a good standard of living are mainly severe malformations and complications of very low birthweight and short gestation period

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Table 2. *Clinical data for 65 infants of non-diabetic mothers*

Infants of non-diabetic mothers	No	Foetal weight	Placental weight	PRBV	Gest. age	Apgar score 1 min	Apgar score 5 min	Clinical data for pregnancy and delivery
Vaginal delivery Early clamping	14	3 034	518	88	39	9	10	Normal pregnancy 13 Threatened abortion 1 Of these foetal distress + vacuum extraction 1 deep transverse arrest 1 hypertonic labour 1 cephalic presentation 13, breech 1
		550–4 200	390–630	50–159	36–41			
Caesarean section Early clamping	10	3 151	593	61	39	7	10	Elective caesarean section 5 previous caesarean section 2, elderly primipara 1 previous difficult delivery 1 stillbirth 1 Emergency caesarean section 5 placenta praevia 2, failed induction 1 disproportion 1 foetal distress 1
		2 510–3 700	355–720	21–108	34–41			
Vaginal delivery Late clamping	38	2 980	535	37	38	9	10	Normal delivery 23 Primary rupture of membranes < 2 500 gm. Bleeding 4. Spont. labour 2. Preeclampsia 3 Oxytocin 4 (medical induction of or uterine inertia)
		1 450–3 910	300–760	3–100	31–41			
Caesarean section Late clamping	3	2 137	437	4	37	8	10	Elective caesarean section 2 elderly primipara 1 preeclampsia (severe) 1 Emergency caesarean section 1 (foetal distress)
		1 410–3 000	290–490	12 39	36–39			
Mean	65	979	536		38			

PRBV – Placental residual blood volume

Table 3 *Mean values for placental residual blood volume in infants of diabetic and non-diabetic mothers in relation to clamping technique and mode of delivery*

	ml/kg body weight		ml/100 gm placental weight		ml PRBV in a child weighing 3 500 g	
	IDM	non-IDM	IDM	non-IDM	IDM	non-IDM
Placental residual blood volume Vaginal delivery Early clamping	39	29	20	17	137	102
Placental residual blood volume Caesarean section Early clamping	23	19	12	10	81	67
Placental residual blood volume Vaginal delivery Late clamping	17	12	9	6	60	42
Placental residual blood volume Caesarean section Late clamping	14	11	8	5	49	39
Real placental transfusion (CS EC CS LC)	9	8	6	5	32	28
Placental transfusion Vaginal delivery (Vag. EC Vag. LC)	22	17	11	11	77	60
Temporary deposited blood (Vag. EC – CS EC)	16	10	8	7	56	35

Table 1 *Clinical data for 58 infants of diabetic mothers*

Infants of diabetic mothers	No	Foetal weight	Placental weight	PRBV	Gest. age	Apgar score 1 min	Apgar score 5 min	White classes A	B	C	D	F	Clinical data for pregnancy and delivery
Vaginal delivery Early clamping	13	3 142 *1 200–4 550*	621 *290–1 050*	126 *25–210*	35 *31–38*	7	9	2	1	6	3	1	Spontaneous labour 5 Vacuum extraction 3 Medical induction of labour 8
Caesarean section Early clamping	29	3 378 1 600–4 700	623 330–955	77 29 139	36 32–38	7	9	1	3	11	9	4	Elective caesarean section 24 Breech 7 previous caesarean section 4 preeclampsia 7 elderly primipara 2, previous stillbirth 2, placental insufficiency 2. Failed induction 3 Foetal distress 1 Spont labour 1
Vaginal delivery Late clamping	8	2 906 1 400–4 000	552 310–755	50 23–102	35 32–38	8	9	1	0	2	5	0	Spont labour 2. Spont. rupture of membranes 4 Medical induction of labour 2
Caesarean section Late clamping	8	3 381 2 550–4 700	563 475–700	42 15–100	37 35–38	6	9	0	2	1	3	2	Elective caesarean section 6 Previous caesarean section 3 breech 1 jaundice of pregnancy 1 preeclampsia 1 Failed induction 1 Prolonged rupture of membranes 1
Mean	*58*	*3 261*	*604*		*36*								

PRBV – Placental residual blood volume.

caesarean section patients received Enibomalsodium NFN (Narcodorm) followed by Succinylcholine and N_2O-O only until the birth of the infant. After birth the infants were weighed either on a Bismer balance or on a spring balance (Fa. Berkel) The placenta was weighed after the remaining blood had run off All clots on the maternal side were removed The umbilical cord and the membranes were weighed together with the placenta.

RESULTS

Fig 1 and Table 3 show the placental residual blood volume (PRBV) in ml/kg body weight in the groups of IDM and non IDM divided up according to the mode of delivery (vaginal or caesarean section) and the clamping technique (early or late) It is seen from Fig 1 that the clamping technique is significant for the PRBV Both IDM and non IDM have a larger PRBV with early clamping after both vaginal delivery and caesarean section than they have with late clamping $p<0.01$ (Wilcoxon Test)

The method of delivery is also significant for the PRBV but only when early clamping is employed. With early clamping after vaginal delivery both IDM and non IDM have a larger PRBV than with early clamping after caesarean section These differences are significant ($p<0.01$)

In addition it is seen that whether the infant's mother is diabetic or non-diabetic is also of significance in that with early clamping after vaginal delivery IDM have a larger PRBV than have non-IDM ($p<0.02$)

Further it is seen that with late clamping the size of the PRBV is similar in both IDM and non IDM independent of the method of

is confirmed by Kjeldsen & Pedersen's findings (8). The larger PRBV in IDM could be because the placental weight, as a percentage of the weight of the infant, is a little larger in IDM than in non-IDM and thus there is a greater vascular capacity in the foetal part of the placenta (respectively 18.77% and 17.49% in this material). This is contradicted to a certain extent by Becker's (2) microscopic observations in that he found that IDM had less branching of the villi than did non-IDM. This probably entails decreased capillary capacity of the foetal part of the placenta.

The placental transfusion, and thus the residual volume, must also be dependent upon the central venous pressure of the neonate. This is raised in adults when there is hypervolaemia (1). If this is also the case in the neonate, then it supports the suggestion that IDM are relatively overloaded at birth. Similarly the larger PRBV of IDM during vaginal delivery with early clamping could be explained by the deposition of a larger volume.

The significance of the clamping technique at caesarean section is still not completely elucidated. It is possible that infants delivered by caesarean section, when the transfer of water from foetus to mother has not yet taken place, could develop a relative hypervolaemia during the placental transfusion, and thereby have an increased risk of RDS. This problem is of great importance for IDM, since about 50% are delivered by caesarean section.

By employing the early clamping technique at vaginal delivery we have the possibility not only to obtain normovolaemic infants, but also for an easy method of venesection which could be of importance for infants of diabetic mothers.

SUMMARY

The influence of the method of delivery and the clamping technique on the placental transfusion is investigated by measurement of the placental residual blood volume in 58 infants of diabetic mothers and in 65 infants of non-diabetic mothers.

It is shown that infants of diabetic mothers delivered vaginally compared with infants of diabetic mothers delivered by caesarean section have a larger placental residual blood volume, if early clamping is employed. This relation suggests that also in infants of diabetic mothers, a temporary deposition of the distribution of the foeto-placental blood volume between the infant and the placenta occurs during vaginal delivery because of the impaired venous backflow to the infant. The difference in the placental residual blood volume is more pronounced for infants of diabetic mothers than for infants of non-diabetic mothers.

Further it is shown that the placental residual blood volume is significant larger in infants of diabetic mothers but only after vaginal delivery with early clamping compared with the same group of infants of non-diabetic mothers.

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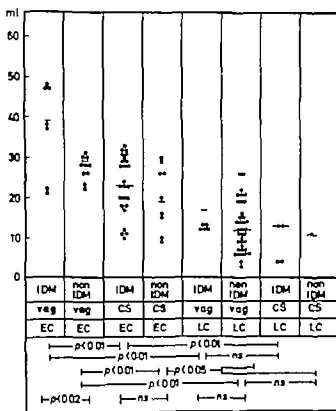


Fig 1 Placental residual blood volume (PRBV) in ml/kg foetal body weight in infants of diabetic and non-diabetic mothers in relation to clamping technique and mode of delivery IDM = infants of diabetic mothers. Non IDM = *infants of non-diabetic mothers.* Vag. = vaginal delivery CS = caesarean section. EC = early clamping. LC = late clamping. Statistical evaluation = Wilcoxon Test.

delivery IDM have on average slightly more PRBV than non IDM but this difference is not significant

From Table 3 it can be seen that the PRBV expressed as a percentage of the placental weight shows on the whole the same relationship as the PRBV expressed in ml/kg body weight, but the significant difference between IDM and non IDM with early clamping after vaginal delivery cannot be found again here

DISCUSSION

On the basis of our findings in monochorionic twins (10) we have set up the hypothesis that also in single pregnancies, there is a temporary displacement in the distribution of the foeto-placental blood volume between the placenta and foetus during the expulsion of the infant at vaginal delivery Thus a temporary hypovolaemia in the foetus and a temporary hyper volaemia in the placenta arises.

Therefore by determination of the PRBV after *vaginal delivery with early clamping* it can be shown that the placentae of these infants contain a larger proportion of the foeto-placental blood volume than the placentae of *infants delivered at caesarean section* with early clamping Similar displacements of the foeto-placental blood volume due to compression of the foetus during the expulsion at vaginal delivery are not expected to occur during caesarean section

These relations have been shown in normal infants (11) The results of determination of PRBV confirm the assumption that IDM follow the same pattern of displacements of the foeto-placental blood volume during vaginal delivery as non IDM Furthermore it is seen that the difference in PRBV in the group of IDM in relation to method of delivery and clamping technique is more obvious and more significant than it is in the corresponding group of non IDM This could be due to the fact that the IDM group although it is a pathological group is a more uniform one with regard to gestational age and method of delivery than the non IDM group since nearly all IDM are delivered in the 37th week at the latest and a large proportion of the caesarean sections are elective (Table 1)

In all groups it is shown that the average PRBV is larger for IDM than for non IDM This difference is, however significant only in the group with early clamping after vaginal delivery If the rest placental blood volume is expressed as ml/kg body weight Although in all groups the PRBV is larger in IDM than in non IDM with late clamping IDM receive a larger placental transfusion than non IDM which is especially obvious after vaginal delivery (PRBV after vaginal delivery with early clamping minus that after vaginal delivery with late clamping and PRBV after caesarean section with early clamping minus that after caesarean section with late clamping) This

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THE INFLUENCE OF THE METHOD OF DELIVERY AND THE CLAMPING TECHNIQUE ON THE RED CELL VOLUME IN INFANTS OF DIABETIC AND NON-DIABETIC MOTHERS

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It has been shown that the clamping technique is of importance for the placental residual blood volume, and for the red cell volume of the newborn infant. With late clamping of the umbilical cord placental transfusion occurs, and increases the blood volume of the newborn infant by 40–60% within a few minutes of birth (6, 9 13 16, 19).

Infants delivered vaginally with early clamping have a significantly larger placental residual blood volume (PRBV) than infants delivered by caesarean section with early clamping (7), thus demonstrating that the method of delivery has likewise influence.

It has been shown by means of PRBV determinations, that infants of diabetic mothers (IDM) receive a placental transfusion, as do infants of non-diabetic mothers (non-IDM), but that the placental transfusion in IDM at vaginal delivery in some cases is larger than that which non-IDM receive under the same conditions (4 8)

There is a connection between the placental transfusion and the blood volume of the infant, since the *foeto-placental* blood volume constitutes the sum of the infant's blood volume and the placental residual blood volume.

The purpose of this work is firstly to describe the influence of the mode of delivery on the red cell volume of the newborn, and secondly to investigate whether the larger placental *transfusion* in infants of diabetic mothers gives rise to a larger red cell volume in the infant.

MATERIAL AND METHODS

Determination of the blood volume was undertaken in 53 neonates, using ^{131}I-labelled albumin as previously described (6).

The red cell volume was calculated from the following formulae:

$$EV_{cor} = BV_{cor} \quad Hct_{cor}$$

$$BV_{cor} = \frac{BV_{m} \quad (100 - Hct_{cor})}{100 - 0.9\, Hct_{cor}}$$

$$\frac{\text{whole body hematocrit}}{\text{large vein hematocrit}} = 0.9$$

trapped plasma factor = 0.95

In 75% of the infants the investigations were undertaken within 3 hours of birth. The remainder were investigated without 24 hours of birth.

N_2O-O_2 or Trichlorethylene (Trilene) was given to provide analgesia before vaginal delivery. All caesarean section patients received Enfluranabodium NFN followed by Succinylcholine and N_2O-O_2.

By early clamping, we mean clamping as quickly as possible; as a rule within 5 sec of delivery and before the child's cry

At vaginal delivery late clamping is carried out after stimulation of the child i.e. after about 2–3 min, and always after the child's cry. At caesarean section, late clamping is carried out about 1 min after delivery

The neonatal course was not considered, but was in most cases uncomplicated.

The material consisted of the two groups of infants as follows:

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dents because of initial bleeding. One of these 2 patients was omitted from the statistical calculation because of foetal bleeding (see footnote to Fig. 1. One patient was induced because of a falling estriol level).

RESULTS

Fig. 1 shows the red cell volume in ml/kg body weight. It can be seen from the figure, that the following 3 factors are significant in effecting the child's red cell volume.

1 *Clamping technique*

When early clamping is performed, the red cell volume of the infant, on the average, is less than when late clamping is performed. This applies to vaginally delivered infants, to those delivered by caesarean section, and to both IDM and non-IDM infants.

IDM vag. EC mean 32 ml/kg
IDM, vag. LC mean 48 ml/kg $p < 0.02$

IDM, CS, EC mean 43 ml/kg
IDM, CS, LC mean 52 ml/kg $p < 0.01$

non-IDM, vag. EC mean 41 ml/kg
non-IDM vag. LC mean 54 ml/kg $p < 0.01$

2. *Method of delivery*

With regard to early clamping infants delivered vaginally have a smaller red cell volume than those delivered by caesarean section.

IDM, vag. EC mean 32 ml/kg
IDM, CS, EC mean 43 ml/kg $p < 0.01$

However the method of delivery has an influence upon the red cell volume only when early clamping is employed, since with late clamping there is no significant difference between the vaginal delivery and the caesarean section groups.

3 *Diagnosis of IDM or non-IDM*

After vaginal delivery with early clamping, IDM have a smaller red cell volume than non-IDM.

IDM vag. EC mean 32 ml/kg
non-IDM vag. EC mean 41 ml/kg $p < 0.02$

With late clamping there is no statistically significant difference in the red cell volume between the groups IDM and non-IDM

Statistical evaluation = Wilcoxon Test. $2a = p$

DISCUSSION

The results of the red cell volume determinations agree on the whole with those values which have been found by other workers (2, 3 12, 14 15 16). On the average our values are a little higher which is probably due to the 10 minute mixing time of the tracer This will systematically give raised blood volume values in neonates, because of their faster albumin disappearance rate (11).

The results support the theory about the influence of the method of delivery on the haemodynamic conditions during delivery In previous articles (5 7 8), we have shown, partly by the determination of the blood volume in monochorionic twins, and partly by determination of the placental residual blood volume, that during the expulsion period of vaginal delivery a change occurs in the distribution of the foeto-placental blood volume, so that the placenta temporarily becomes hypervolaemic, while the infant's blood volume is correspondingly reduced.

These changes in the foeto-placental blood volume are illustrated with the red cell volume, since this does not alter appreciably over the first 24 hours after delivery (15 16).

The distribution of the foeto-placental blood volume between foetus and placenta during caesarean section and vaginal delivery illustrated by the red cell volume determinations, has been shown for IDM only With regard to both the clamping technique and the method of delivery IDM follow the same haemodynamic pattern as non-IDM, as illustrated by means of the rest placental blood volume (8) The red cell volume after late clamping is the same in both IDM and non-IDM, just as no difference can be demonstrated in the red cell volume after late clamping between

Table 1 *Clinical data and mean erythrocyte volume in ml/kg body weight in 35 infants of diabetic and in 18 infants of non-diabetic mothers in relation to clamping technique and mode of delivery*

RCV = Red cell volume

	No	Fetal weight	Gest. age	RCV (ml/kg)
Infants of diabetic mothers				
Vaginal delivery Early clamping	7	2 846 (1 200–4 550)	35 (31–38)	32 (28–40)
Caesarean section Early clamping	15	3 361 (2 450–4 700)	36 (32–38)	43 (35–49)
Vaginal delivery Late clamping	5	2 980 (1 400–4 000)	34 (30–38)	48 (37–66)
Caesarean section Late clamping	8	3 381 (2 550–4 200)	37 (35–38)	57 (43–63)
	35	3 208	36	44
Infants of non-diabetic mothers				
Vaginal delivery Early clamping	10	2 155 (1 300–2 900)	35 (31–39)	39 (22–48)
Vaginal delivery Late clamping	8	2 441 (1 750–3 500)	36 (31–40)	54 (37–66)
	18	2 282	35	46

Infants of diabetic mothers (IDM)

Thirty-five infants, of whom 12 were delivered vaginally and 23 by caesarean section. Early clamping was carried out in 22 and late clamping in 13 infants.

Gestational age and weight are shown in Table 1

White class (17) distribution was.

A	B	C	D	F
3	5	12	8	7

See Table 2.

Apart from the mothers diabetes, pregnancy was uncomplicated in 26 cases. 7 patients had pre-eclampsia and 1 patient experienced bleeding in pregnancy Spontaneous labour occurred in 8 patients, and 7 patients were induced with oxytocin

Table 2 *White s classification (1965) as applied in the Rigshospital (12)*

	Onset (years)		Duration (years)	Retinopathy benign
A	Chemical diabetes (diet ± oral drugs)			
B	> 20	and	< 10	absent
C	10–19	or	10–19	absent
D	< 10	or	> 20 or	present
F	Nephropathy and/or proliferative retinopathy			

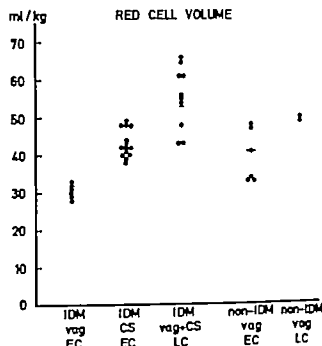


Fig 1 Scattergram of red cell volume in ml/kg body weight in newborn infants of diabetic and non-diabetic mothers following early and late clamping of the cord in relation to vaginal delivery or caesarean section. o represents a newborn infant who had hemorrhagic amniotic fluid. This infant had the lowest Hct and required a transfusion. The data for this case have not been included in the calculation of any of the means or statistical descriptions. IDM = infant of diabetic mother non-IDM = infant of non-diabetic mother vag. = vaginal delivery CS = caesarean section EC = early clamping, LC = late clamping

There were 20 elective caesarean sections because of spontaneous labour before the predetermined section date

The 3 remaining caesarean sections were done because of labour or spontaneous rupture of the membranes in patients who should have done elective sections.

Infants of non-diabetic mothers (non IDM)

Of the 18 infants early clamping was undertaken in 10 and late clamping in the remaining 8 In this material determination of the red cell volume was only carried out in infants with on the average, the same gestational age as IDM. Elective caesarean section in the 37th week is so rarely carried out in non-diabetic mothers, that in this series, only vaginally delivered infants were included

Pregnancy was complicated by pre-eclampsia in 2 cases, and by bleeding in 5 cases.

Labour began as a result of spontaneous rupture of the membranes in 6 patients, with spontaneous contractions occurring in 9 patients, and in 2 pa

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vaginal delivery and caesarean section in normal patients (15) This may also be assumed to occur after early clamping in non IDM delivered by caesarean section in the same manner as in IDM

By determinating the placental residual blood volume we have been able to show that during vaginal delivery IDM deposit a larger proportion of the foetal blood volume in the placenta than non IDM (8) This relationship is confirmed in that investigation where it is shown that after vaginal delivery with early clamping IDM have a lower red cell volume in comparison to non-IDM ($p<0.02$, Fig 1)

With late clamping no difference in the red cell volume was found between IDM and non-IDM Neither did the method of delivery have any influence on the red cell volume with late clamping These relationships correspond completely to those found in the placental residual blood volume (8) where no differences can be shown under the same conditions. This result also corresponds to the results reported by Steele (15) who showed that in normal neonates there was no difference in the red cell volume between infants delivered vaginally and those delivered by caesarean section when late clamping was performed.

In our opinion the results and theoretical considerations are of some practical clinical importance since there are several circumstances which suggest that IDM are relatively hypervolaemic at delivery It is a clinical observation that IDM never appear to be anaemic even when very early clamping is performed, as opposed to non IDM Furthermore, in the neonatal period, IDM reduce their plasma volume more than non-IDM although in the two groups IDM and non IDM, with late clamping there is no significant difference in the red cell volume in ml/kg body weight (5) Since IDM have relatively more fat tissue than non-IDM (10) and thus probably reduced vascular capacity (1) these relationships suggest a relatively hypervolaemia at delivery

Since during delivery vaginally delivered infants deposit a relatively large part of their blood volume in the placenta as mentioned, early clamping in these infants will act as a venesection While this may be advantageous for infants of diabetic mothers its value for other categories of infants is still unknown.

Finally it must be remarked that in the evaluation of the blood volume and the red cell volume of the neonate, one should consider not only the clamping technique but also the method of delivery

SUMMARY

The red cell volume has been investigated in 35 infants of diabetic mothers and in 18 infants of non-diabetic mothers. It is shown *that not only the clamping technique* but also the method of delivery has an influence on the red cell volume of the infant. Thus the red cell volume is less in infants delivered vaginally with early clamping compared with infants delivered by caesarean section with early clamping.

These findings support the theory that during vaginal delivery a temporary change in the distribution of the foeto-placental blood volume between placenta and child occurs.

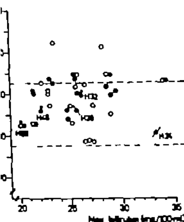
It is suggested that IDM during vaginal delivery with early clamping deposit more blood in the placenta than do the non IDM since we found a smaller red cell volume per kg body weight in the IDM under these circumstances.

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..g *1* IQ plotted against maximal bilirubin values in ..5 children in the ABO-compatible group. ● = Exchange transfusion. ○ = No exchange transfusion. H 24 = Case No. – Sensorineural hearing defect. – Coordination disturbance. *P* = Psychological disturbance.

..een described in detail earlier by Killander et al. (18).

Table 2 shows the number of exchange transfusions in the two hyperbilirubinaemia subgroups. The distribution of the transfused infants among the different blood groups in the ABO incompatible group can be seen in Table 1

The *control group* was selected as follows: For each infant in the hyperbilirubinaemia group the nearest preceding or following infant of the same sex and fulfilling the same selection criteria but with no visible jaundice and with no record of a bilirubin value above 10 mg/100 ml was chosen.

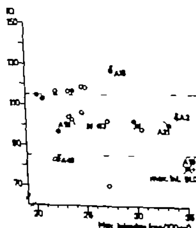


Fig 2. IQ plotted against maximal bilirubin values in 45 children in the ABO-incompatible group [ ] = No intelligence test performed. Other symbols as in Fig. 1.

Table 1 *ABO-incompatible group Blood group distribution among mothers and children, reticulocytosis over 4% from the 3rd day of life and the number of exchange-transfused children*

Mother's blood group	Child's blood group	No. of children	Reticulocytosis	No. of transfused children
O	A	27	10	13
O	B	8	3	4
A	B	3	1	0
A	AB	1	0	0
B	A	3	1	2
B	AB	3	1	1
Total		45	16	20

Laboratory methods. All bilirubin determinations were performed in the laboratory of the Department of Paediatrics as part of the routine work. The values are expressed as total bilirubin. The maximal direct bilirubin was 2.0 mg/100 ml. During 1957 the serum bilirubin concentration was determined by the method of *Jendrassik & Grof* (14). From the beginning of 1958 to the end of 1961 the method of Malloy & Evelyn (19) was used, and in 1962 a modification by Michaëlsson (21) of the Jendrassik–Grof Nosslin method was introduced. The latter has the smallest error of the method and is not influenced significantly by haemolysis in the sample (22).

The haemoglobin concentration and the reticulocyte count were determined by the routine methods. Vitamin K was given only in the natural form, in a dose of 1–2 mg. No streptomycin was given neonatally

METHODS

The age range of the children at the time of the follow-up examinations was 6½ to 13 years. The

Table 2. *Number of exchange transfusions in the two hyperbilirubinaemia subgroups*

The difference in exchange transfusion frequency is not statistically significant

Transfusions per child n	ABO-compatible group (66 children)	ABO-incompatible group (45 children)
1	22	14
2	2	4
3	0	1
4	0	0
5	0	1
Total no. of transfused children	24/66 (36.4%)	20/45 (44.4%)
Total no. of transfusions	26	30

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A FOLLOW UP STUDY OF HYPERBILIRUBINAEMIA IN HEALTHY FULL-TERM INFANTS WITHOUT ISO IMMUNIZATION

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It is well documented that damage to the central nervous system can occur in the newborn as a result of hyperbilirubinaemia in association with haemolytic disease and prematurity The level of hyperbilirubinaemia that can be tolerated in the healthy full term infant without iso-immunization is, however still uncertain (3 4 6 8 11 12 13 17 18 23 24 25 27) The aim of this investigation was to elucidate this question by a detailed follow up of 226 children half of whom had had neonatal hyperbilirubinaemia with a maximal bilirubin value exceeding 20 mg/100 ml and the other half of whom had had no clinical jaundice

MATERIAL

The follow-up investigation comprised a total of 226 infants born between 1957 and 1962. These were divided into two groups, a hyperbilirubinaemia group of 111 infants (77 boys and 34 girls), and 115 controls. Of the originally selected infants, 6 in the hyper bilirubinaemia group (5 4%) and 2 in the control group (1 7%) were not available for examination, primarily because they lived too far away All of the infants had been cared for in the nursery and/or paediatric section of the Uppsala University Hospital in the neonatal period but a few had been born in other hospitals and had been transferred to the University Hospital during their first days on account of hyperbilirubinaemia.

The *hyperbilirubinaemia group* consisted of all healthy full-term infants who during the years 1957–1962 had had maximum bilirubin levels exceeding 20 0 mg/100 ml. Thus all infants with any form of iso-immunization (defined as positive direct Coombs test), obstructive jaundice, sepsis, asphyxia or other serious neonatal disorders were excluded. Also excluded were infants born to mothers having prolonged labour a difficult delivery Caesarian section or any other major complication of pregnancy labour or delivery Only infants weighing 2 500 g or more at birth and born during the 38th to 42nd weeks of pregnancy were included.

The hyperbilirubinaemia group was divided into two subgroups: ABO-compatible and ABO-incompatible.

The *ABO-compatible group* consisted of 66 infants whose blood group was compatible with that of the mother The maximal bilirubin values in this group ranged between 20.0 and 34.8 mg/100 ml (Fig. 1).

The *ABO-incompatible group* consisted of 45 infants whose blood group was incompatible with that of the mother (Table 1) Their maximal bilirubin values ranged between 20 0 and 51 0 mg/100 ml (Fig. 2).

Among the 45 infants in the ABO-incompatible group there might have been some unrevealed cases of mild erythroblastosis despite a negative direct Coombs test. For example, 16 infants had a reticulocyte value of more than 4% on the third day of life or later In 13 of these 16 cases the mother's blood group was 0 and the child's either A or B (Table 1).

During the years 1957–1962 the following principles were applied when considering the indications for exchange transfusion. In full-term infants with hyperbilirubinaemia but with no signs of erythroblastosis the indirect bilirubin level should be prevented from exceeding 30 mg/100 ml and in infants with possible erythroblastosis (as a result of A or B incompatibility) it should not exceed 25 mg/100 ml. Infants with signs of neurological disturbances or a poor general condition were transfused at lower bilirubin levels. In several infants, however the maximal bilirubin level exceeded the values mentioned above. In some of these cases the infant already had a high value on admission from another hospital in other cases the infant had been included in a previous study in our Department, in which every alternate infant was given an exchange transfusion and the others were not, if their clinical condition was satisfactory (18) The technical procedure in exchange transfusions has

ble 3 *Summary of results*

ι. of ldren	Sex M(ale) F(emale)	Case no.	Coordination disturbance	Neurogenic hearing loss	IQ < 85	Psychological disturbance
IO-compatible group (*N* = 66)						
				–	–	–
	M	H 24			–	–
	M	H 28	–			–
			–	–	7	
	M	H 32	–	–	–	
	M	H 48	–	–	–	
	F	H 59	–	–	–	
BO-incompatible group (*N* = 45)						
						–
	M	A 19			–	–
	M	A 21	–		–	–
	M	A 51	–			
	F	A 49	–	–		
			–	–	3	–
	M	A 2	–	–	–	
	M	A 15	–	–	–	
Total hyperbilirubinaemia subgroups (*N* = 111)			2	4 (3 7 %)	12 (10.8 %)	6 (5 4 %)
Control group (*N* = 115)						
1	M	K 23		–		
1	M	K 3	–	–		
9			–	–	9	–
1	F	K 71	–	–	–	
Total control group			1	0	11 (9 6 %)	3 (2.6 %)

ordination (Table 3). One of these children (case A 19) exhibited classic symptoms of kernicterus, with pronounced choreoathetosis and severe bilateral hearing impairment. The other child (H 24) had ataxic athetosis, a slight speech disorder perceptional disturbances and a slight behavioral abnormality Among the 115 children of the *control group* there was one 11 year-old boy (K 23) with slight unilateral incoordination mainly affecting the left arm and hand (Table 3) This child also had a low level of intelligence and had undergone psychiatric investigation. The overall symptoms of this patient pointed to the probability of slight brain damage but there was no indication of a cerebral lesion either in the medical history or in the delivery record.

Hearing examination

Of the 226 children in the investigation, 223 underwent audiometric examination. For organizational reasons 3 children in the hyperbilirubinaemia group were not examined, but there was no suspicion of hearing impairment in any of them.

Taking as a criterion of sensorineural hearing loss a reduction in hearing of at least 30 dB at two frequencies between 500 and 6 000 c/s in the same ear there were 4 children (3 7 %) with such loss among the 108 children in the *hyperbilirubinaemia group* who underwent audiometric examination (Figs. 1 2 and 3 Table 3) One of these children (A 19) also had choreo-athetosis, while the other three exhibited no signs of motor impairment (Table 3).

No cases of sensorineural hearing loss were found among the 115 children of the *control group* The difference between the two groups in this respect was not statistically significant, however

Psychological examination

An intelligence test was administered to 108 of the 111 children in the hyperbilirubinaemia group Three children could not be tested be

examining physicians had no knowledge of whether the children belonged to the hyperbilirubinaemia group or the control group thus the case history was not taken until the examinations had been completed A predetermined routine was followed at the ex aminations. In order to ensure standardization in taking the medical history and in the evaluations in the physical and neurological examinations, the authors went through these steps together on some ten children at the beginning of the investigation. All children except four were examined by one or other of the authors the other four were examined by other paediatricians.

Medical history

A careful medical history was taken from the parents of each child with special attention to heredity home environment, school situation, physical and mental development, somatic disorders and mental health.

Physical examination

This comprised a complete routine examination.

Neurological examination

This consisted of three parts. general neurological ex amination, and tests of the gross and fine motor functions.

General neurological examination This comprised conventional testing of the function of the cranial nerves, gross testing of the sensory system, evaluation of the muscle tonus and examination for the presence of paresis, hyperkinesia atrophy etc. The biceps, abdominal quadriceps and Achilles tendon reflexes were tested bilaterally as well as the Babinski sign

Gross motor functions were examined by the following tests: walking with open eyes, walking with closed eyes, standing on tiptoes, standing on the heels, Romberg's test, jumping 10 times to a given rhythm while putting one leg alternately in front of the other walking straight along a 4-metre-long line hopping on each leg along a 4-metre long line; marching to a given rhythm while at the same time making a circular movement first with one arm and then with the other with eyes closed holding the arms stretched out in front, supinated in the vertical plane, for 20 seconds alternating pronation and supination of the hands for 20 seconds, measurement of the gross strength of each hand with a dynamometer

Fine motor functions were examined by the following tests: testing of the function of the ulnar radial and median nerves; using one hand at a time, moving small pieces with precision to different holes in a game standing without support while cutting out a circle 5 cm in diameter drawn on paper picking up small metal clips with each hand in turn, and holding them in the hand until about 10 had been picked up with the eyes closed, touching the tip of the nose with first the right and then the left index finger

Hearing examination

The hearing was tested with a standard, pure tone audiometer calibrated according to the ISO recommendation R 389 and covering the frequencies 125-8 000 c/s. The examinations were carried out and the evaluations made at the Department of Audiology in Uppsala.

Psychological examination

Individual intelligence testing of each child was conducted by a qualified psychologist. Children of ages 8 13 years were tested with WIT which is a test, built up of five subtests, verbal and non verbal (29). The IQ was calculated from the combined results of the five different subtests. Children of ages 6½-7 years were given the Terman-Merrill test (76).

The diagnosis "psychologically disturbed was made by the authors when taking the history and carrying out the physical examination, special consideration being paid to the functional adaptation at home at school and to classmates. This diagnosis was given only to children with both noteworthy symptoms and functional maladjustment. Examples of symptoms observed in children with psychological disturbances were anxiety with psycho-somatic manifestations, phobic reactions, contact difficulties, problems with classmates and aggressiveness. The symptoms were sometimes combined with enuresis and encopresis. A few children had played truant from school and had shown other types of asocial behaviour

Statistical methods

Conventional statistical methods, which may be found in an ordinary statistical textbook, were used in the investigation.

To test the difference between two proportions, Fisher's exact test was used when the samples were small. For larger samples a normal approximation was used

The correlation coefficient, r between the variables x and y is calculated as

$$r=\frac{\Sigma xy-\frac{\Sigma x\,\Sigma y}{n}}{\sqrt{\left(\Sigma x^2-\frac{(\Sigma x)^2}{n}\right)\left(\Sigma y^2-\frac{(\Sigma y)^2}{n}\right)}}$$

where n is the number of observations.

To test the hypothesis of zero correlation the following formula was used

$$t=r\sqrt{\frac{n-2}{1-r^2}}$$

Under certain assumptions, and when the hypothesis holds, this variate has Student's distribution with n-2 degrees of freedom.

RESULTS

Medical history physical and neurological examinations

Among the 111 children of the *hyperbilirubinaemia group* there were 2 with athetotic inco-

Since then examined regularly. Motor development retarded, stood up at 14 months, started walking at 18 months. At 2½ years choreo-athetosis. From 4–5 years it became clear that hearing was impaired. At follow-up at 7½ years he showed severe choreo-athetosis and advanced bilateral neurogenic hearing loss (Fig. 3). Ocular movements normal. IQ difficult to evaluate because of deafness, but repeated tests with the Leiter test battery gave a value of about 79. According to his parents his choreo-athetosis is not a severe handicap, but his deafness is extremely disabling despite a hearing aid and special training.

Case H 24

Boy 2nd child of healthy parents. Pregnancy and delivery normal. Birth weight 3 790 g. Mother and child A Rh+. Coombs direct test negative. Jaundiced on the day after delivery: bilirubin 9.4 mg/100 ml, Hb 20.4 g/100 ml, reticulocytes 8.2% and 8.0% on the 4th day. Bilirubin maximum of 33.2 mg/100 ml on the 5th day when an *exchange transfusion* was given. This reduced the bilirubin level, but up to the 9th day it was constantly above 23 mg/100 ml, on the 13th day it was 17.0 mg/100 ml. General condition good throughout this period. At 3 years a moderate ataxia of the legs and trunk was observed. Hearing normal at play audiometry and EEG normal.

On follow-up at 7½ years he exhibited a moderate coordination disturbance of the ataxia-athetosis type, a moderate speech disorder with incorrect word construction and defective pronunciation, perceptional abnormalities and a slight behavioral disturbance with agitation and anxiety, not, however, of such an extent that he was given the diagnosis "psychologically disturbed". IQ 91. Audiogram normal.

Case A 21

Boy 2nd child of healthy parents. Sibling healthy. Pregnancy and delivery normal. Birth weight 3 530 g. Jaundice on 2nd day: bilirubin 15.3 mg/100 ml, Hb 21.7 g/100 ml, spleen slightly palpable. Reticulocytes 8.4% on 4th day. Mother's blood group O Rh–, child's A Rh–. Direct Coombs test negative. In mother's blood an anti-A agglutinin, titre 1/2048, which reacted against the child's blood cells in vitro. Exchange transfusions on 4th day when bilirubin was 23.4 mg/100 ml, on 6th day when the peak bilirubin was 33.2 mg/100 ml and on 7th day when the value had again risen to 31.1 mg/100 ml. The bilirubin level then fell rapidly. On the 6th day the child appeared very sleepy but otherwise in normal condition. At follow-up at 6 weeks, 3 and 14 months nothing abnormal. On further examination at 2 years 7 months it was noted that the child had first spoken at 18 months and had first spoken a sentence at 2 years 4 months. Hearing impairment was not suspected, however. On follow-up at 11 years bilateral neurogenic hearing impairment was found (Fig. 3). Otherwise normal. The hearing impairment has caused no serious difficulties and he does not need a hearing aid. Audiometry on the father revealed a slight, very frequency-limited hearing loss in the upper frequencies—probably noise damage. Audiometry on the mother revealed a slight hearing loss in the lower frequencies but otherwise nothing abnormal.

Case A 51

Boy 1st child of healthy parents. Pregnancy and delivery normal. Birth weight 3 630 g. Jaundice on 2nd day. 3rd day: bilirubin 21 mg/100 ml, Hb 23.8 g/100 ml and reticulocytes 7.2%. Mother's blood group O Rh+, child's A Rh+. Direct Coombs test negative. An anti-A agglutinin of the immune type, of low titre, was found in the mother's blood, but this did not react with certainty against the child's blood cells in vitro. From the 3rd to the 10th day the child's bilirubin was over 21 mg/100 ml, maximal value 23.7 mg/100 ml on the 7th and 10th days. No exchange transfusion. On 12th day bilirubin had decreased to 18.5 mg/100 ml. General condition good throughout the neonatal period. At follow-up at 8 years the child showed bilateral neurogenic hearing loss, more pronounced on the left side (Fig. 3), but otherwise his condition was normal. No history of deafness in the family. Mother's audiogram normal. Audiometry on the father was not possible, owing to illness.

Case H 28

Boy 1st child of healthy parents. Pregnancy and delivery normal. Birth weight 3 630 g. Jaundice on 3rd day. Maximal bilirubin value 25.6 mg/100 ml on 4th day. Hb 23.9 g/100 ml, reticulocytes 3.4%. After exchange transfusion on the 4th day the bilirubin level fell rapidly. General condition normal throughout the neonatal period and nothing abnormal neurologically. No evidence of blood group incompatibility. Mother and child O Rh+. Direct Coombs test negative. No irregular antibodies in the mother's serum. At the first follow-up at 2½ years it was noted that the child had first spoken sentences at 2 years 4 months; otherwise nothing of note in the history or general condition.

At 3 years 4 months the child was run over by a moped and sustained a fracture of the left tibia. No vomiting or unconsciousness. No head X-rays were taken. Two days after the accident the mother thought that he did not react as previously when spoken to, and 2 days later he was admitted to a department of otorhinolaryngology for investigation. X-rays of skull and ears normal. No dizziness, nystagmus or disorders of balance. The boy's speech and tone of voice appeared to be normal, but psychologically he was apprehensive and it was difficult to communicate with him. Audiometric tests indicated hearing impairment. At follow-up at 10½ years he was found to have severe bilateral hearing loss (Fig. 3) and rather poor speech. He used a hearing aid. His IQ was 102 and in all other respects he appeared normal.

The aetiology of this patient's deafness is not clear. Probably not due to hereditary factors. Mother has a normal audiogram and father has an occupation (air force pilot) that demands completely normal hearing. The hearing impairment was first noted after the

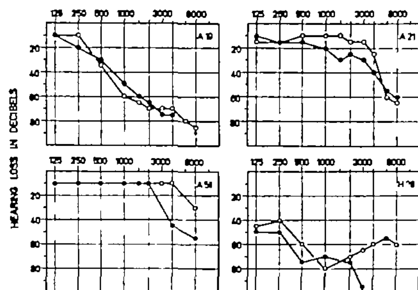


Fig 3 Audiogram from 4 children (*A 19 A 21 A 51 and H 28 see text*) with sensorineural hearing defects.

cause of organizational difficulties but all three gave the impression of average intelligence All children in the control group were tested The mean values and standard deviations of the IQs in the two hyperbilirubinaemia subgroups and the control group are presented in Table 4 Statistical analysis revealed no significant difference between the groups The individual IQ values of the ABO-compatible group plotted against the maximal bilirubin values, can be seen in Fig 1 and the corresponding values of the ABO-incompatible group in Fig 2. On statistical analysis no significant correlation was found between the IQ values and the maximal bilirubin values in either of these groups

Psychological disturbances were found in 6 (5 4%) of the children in the hyperbilirubinaemia group and in 3 (2.6%) of those in the control group (Table 3) This difference is not statistically significant. No significant correlation was found between the maximal bilirubin values and the presence of psychological disturbances (Figs. 1 and 2) The results are summarized in Table 3

Case Reports

Case A 19

Boy 1st child of healthy parents. Mother previously two spontaneous miscarriages. Pregnancy and delivery normal Birth weight 3 260 g. Jaundice on 2nd day Referred from another hospital on 3rd day with bilirubin 24 7 mg/100 ml, Hb 14.8 g/100 ml and reticulocytes 1.8% rather lethargic but otherwise in good condition. Mother's blood group O Rh+ child's B Rh+ Direct Coombs test negative Exchange transfusion on 3rd day with O Rh+ blood. Bilirubin before 23.8 mg/100 ml and after 12.4 mg/100 ml. During following night a rapid, unexpected bilirubin increase to 51 mg/100 ml, but still no apparent effect on general condition or neurological status. Extensive serological investigations of both mother and child did not reveal the reason for the sudden bilirubin increase. Direct Coombs test negative both *before* and after the first exchange transfusion. No irregular antibodies in the mother's serum either by indirect Coombs test or an enzyme technique. In mother's serum an anti-B agglutinin of the immune type (titre unknown) Altogether 5 exchange transfusions in the 3rd to 5th days, and bilirubin level fell to about 15 mg/100 ml. On 4th day symptoms of brain damage in form of hypertonia of the arms and shrill crying. No convulsions or tendency to opisthotonus. Moro reflex normal the whole time. Muscle tone normal after 4 days and the cry after about 6 days.

Table 4 *IQ in the hyperbilirubinaemia and control groups*

	No of children tested	Mean IQ	Variance	Standard deviation
ABO-compatible group	66	106.6	297.82	17.26
ABO-incompatible group	42	105.9	239.84	15 49
Control group	115	105.9	280.83	16.76

serological explanation and the possibility that the *donor* blood was already haemolysed can neither be proven nor excluded. Case H 24 had both a high maximal bilirubin value 33.2 mg/100 ml, and a protracted high bilirubin level—over 23 mg/100 ml for 6 days.

Sensorineural hearing defects

It is well documented that haemolytic disease of the newborn may be associated with sensorineural hearing loss. Crabtree & Gerrard demonstrated in 1950 that deafness was present in 16 out of 20 cases of athetoid cerebral palsy (5). In Sweden a similar figure has been reported by Barr & Klockhoff (2) among a series of children with athetosis they found that 70% had neurogenic hearing damage. The development of sensorineural hearing loss has also been described in infants with haemolytic disease of the newborn due to A or B immunization (8, 15), and in premature infants with hyperbilirubinaemia without iso-immunization (8, 16). The sensorineural hearing loss associated with haemolytic disease of the newborn can occur without simultaneous athetosis or other neurological damage (15) On the other hand, hearing loss associated with neonatal hyperbilirubinaemia in otherwise healthy full-term infants without iso-immunization has not, to our knowledge, been described previously with certainty (3 8, 12, 15 16 17 23)

The cause of the sensorineural hearing loss in haemolytic disease of the newborn is believed to be deposition of bilirubin in the cochlear nuclei (8, 16) but damage to the cochlea has also been considered a possibility (9). The hearing loss mainly affects the higher frequencies, with a maximum degree of impairment in the range of 2 000 to 4 000 c/s (8, 16) As a rule the hearing loss is approximately equal on both sides (16), but unilateral deafness has also been described (15).

From routine auditory examinations of school children in Sweden it is known that sensorineural hearing loss—of different causes—occurs in a frequency of about 0.7 to 0.9% at school ages, with a strong predominance of boys (20). These figures do not include children attending special schools for the deaf In boys the frequency of sensorineural hearing loss increases during school age (20). In a large group of 14-year-old Swedish children a frequency of about 2.1% was found (1). In cases with conductive hearing loss no sex difference was observed, whereas sensorineural defects were almost twice as common in boys (1).

Our control material is too small to allow any generalized statements concerning the frequency of sensorineural hearing loss, but with such a selection of healthy infants it should be lower in this group than the frequency found in a general school examination. It is interesting, however to compare the control material with our hyperbilirubinaemia group—selected by the same criteria apart from the high bilirubin level, in the latter group there are 4 cases of sensorineural hearing loss, all boys, as compared with none among the control group. This difference is not statistically significant, however By excluding other neonatal complications than hyperbilirubinaemia and by checking the parents' hearing, an attempt was made as far as possible to exclude other causes of sensorineural hearing loss. The difference between the hyperbilirubinaemia and the control group indicates, therefore, that sensorineural hearing loss can be caused by neonatal hyperbilirubinaemia even in otherwise healthy full-term children without haemolytic disease of the newborn

Intelligence level

A lowered IQ in deeply jaundiced children with haemolytic disease of the newborn has been reported by some authors (7 28). It now seems to be fully established, however from several investigations, that non-haemolytic hyperbilirubinaemia of a moderate degree does not affect the IQ (3 6, 24 27) This conclusion is supported by the present study in which no difference in IQ was found between the hyperbilirubinaemia and the control group. Disturbances in cognitive functions such as learn-

accident at the age of just over 3 years, but previous to this it had been observed that his speech was retarded and he first spoke a sentence at 28 months. Further audiometric tests have indicated that his hearing impairment may be localized to the organ of Corti.

Case K 23

Boy 2nd child of healthy parents. Siblings healthy Pregnancy and delivery normal Birth weight 4 120 g. Nothing abnormal in the neonatal period At 3 years he became aggressive, had fits of rage and showed other behavioral disturbances. At 8 years investigated at a department of child psychiatry where a low intelligence level a pathological EEG and a behavioral aberration was found At follow-up at 11 years slight incoordination in the left arm and hand was also revealed IQ at that time 81 Audiogram normal

DISCUSSION

The question of the risk of damage after neonatal hyperbilirubinaemia in healthy full term infants without iso-immunization has not been answered unequivocally in the literature Many reports do not differentiate between full term and premature infants or between hyperbilirubinaemia due to Rh immunization due to ABO immunization and without iso-immunization Control groups are often lacking and follow up examinations are sometimes carried out at much too early an age so that hearing impairment for example cannot have been excluded with certainty

Mores et al (23) concluded in 1959 that hyperbilirubinaemia without iso-immunization does not involve a risk of brain damage in healthy full term infants and that exchange transfusion is unnecessary in hyperbilirubinaemia unless iso-immunization is present In 1961 Bjure et al (3) found among 113 children no cases of brain damage that could be attributed to hyperbilirubinaemia alone, but three children with cerebral damage had all had maximal bilirubin values above 20 mg/100 ml. In 1968 Holmes et al (12) reported 63 healthy full-term infants with neonatal bilirubinaemia of varying causes, who showed no evidence of neurological abnormalities or perceptive hearing loss In two publications in 1960 and 1963 Killander et al. (18 17) described 94 full term infants who were followed up to the ages of 24–32 months without any signs of cerebral damage being found. Seventeen of these children are also included in the present investigation and 2 of them who on examination at 24–32 months showed no signs of damage were later found to have sensorineural hearing impairment (Cases A 21 and H 28) In their first report (18) Killander et al also mentioned 3 cases of kernicterus among 18 full-term infants who had had deep jaundice of the "physiological" type during 1950–55 Kernicterus in full term infants has also been reported by Zuelzer & Mudgett (31) they found nine full term infants among 32 cases of kernicterus not associated with Rh immunization

Coordination disturbances

Our study has concerned as far as possible, a homogeneous group of patients, full-term infants with respect to gestational age and birth weight, with serum bilirubin values exceeding 20 mg/100 ml and no signs of iso-immunization and otherwise healthy in the neonatal period

It is clear from this study that damage resulting from hyperbilirubinaemia occurs also in this patient group In the 2 children with athetotic incoordination (A 19 and H 24) it seems highly probable that this condition was due to hyperbilirubinaemia both had had high bilirubin values in the neonatal period, and no other explanation for the symptoms was found The only child in the control group who had any form of coordination disturbance (K 23) had a different pattern of symptoms a very slight, *unilateral* incoordination in the arm and hand and the picture was dominated by mental retardation a pathological EEG and behavioral aberration The development of distinct kernicterus in Case A 19 is fully explainable by the high bilirubin level, 51 mg/100 ml, which had been present for at least 12 hours. The reason for the rapid haemolysis after the first exchange transfusion is not clear however There appeared to be no

and psychological tests. Among the 111 children with neonatal hyperbilirubinaemia 2 had coordination disturbances of the athetotic type and 4 had sensorineural hearing impairment, while none of the 115 control children had such disorders. The differences in the frequencies of these conditions were not statistically significant, however. Neither did the frequencies of subnormal intelligence and psychological disturbances in the two groups differ significantly. Exchange transfusion is recommended *in healthy full-term infants* without iso-immunization as a measure to prevent the serum bilirubin level from exceeding 25 mg/100 ml.

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ing, memory and adaptive behaviour, on the other hand, have been described recently by Odell et al (24) No attempt was made in the present study to evaluate the cognitive functions in the same way as Odell et al. but the WIT test used here includes subtests with spatial factors A special perusal of the results of these subtests revealed no difference between the two groups, however and thus no indication of an increased occurrence of disturbances in cognitive functions of the cerebral lesion type in the group with neonatal hyperbilirubinaemia

Psychological disturbances

There was no statistically significant difference between the frequencies of psychological disturbances found in the two groups—5 4% in the hyperbilirubinaemia group and 2.6% in the control group The relatively low frequency figures should be considered in the light of the stringent criteria used in this study for the diagnosis "psychological disturbance" where symptoms of behavioral disturbance as well as a functional maladjustment of the child to its environment were required Taking this into account our frequencies of "psychological disturbances" correspond fairly well with the figures published from a general health survey of 4-year-olds in Sweden where in one series of examinations marked aberration" was found in 3% (10) and in another series 3 6% were referred to a psychiatrist and/or a psychologist (30)

CONCLUSIONS

From the results of the present investigation it is still not possible to state definitely the indications for exchange transfusion in healthy full term, newborn infants with hyperbilirubinaemia not due to iso-immunization The relatively small number of infants with high bilirubin values may explain the lack of statistically significant differences It seems clear however that a high bilirubin level led to complete symptoms of kernicterus in one child (A 19) and to athetotic incoordination in another (H 24) Furthermore the occurrence of 4 cases of sensorineural hearing loss among the children of the hyperbilirubinaemia group, while there were none in the control group would seem to point to a high probability that this was due to the elevated bilirubin level. Thus altogether 5 children from the hyperbilirubinaemia group exhibited marked deviations such as are usually associated with damage caused by bilirubin whereas none of the children in the control group showed such disorders.

Since all children with coordination disturbances and/or hearing impairment in this study had had maximal bilirubin values above, or in one case in the vicinity of 25 mg/100 ml, it would seem reasonable that in healthy full-term infants with no signs of iso-immunization the bilirubin level should not be allowed to exceed this value

A special problem is encountered in infants with possible A or B immunization, i.e. usually infants with blood group A or B of mothers with blood group O and in whom early jaundice and/or pronounced reticulocytosis mean that erythroblastosis due to A or B immunization cannot be excluded despite a negative direct Coombs test. These infants should probably be treated initially as cases of erythroblastosis and should not be permitted to have unconjugated bilirubin values above 20 mg/100 ml.

SUMMARY

With the aim of shedding light on the indications for exchange transfusion in healthy full term infants without iso-immunization a detailed follow-up investigation was made of 226 children, of whom 111 had had neonatal hyperbilirubinaemia with maximal bilirubin values of 20–51 mg/100 ml and the other 115 (controls) had had no visible jaundice neonatally

The children underwent physical and neurological examinations as well as audiometric

nd psychological tests. Among the 111 children with neonatal hyperbilirubinaemia 2 had coordination disturbances of the athetotic type and 4 had sensorineural hearing impairment, while none of the 115 control children had such disorders. The differences in the frequencies of these conditions were not statistically significant, however. Neither did the frequencies of subnormal intelligence and psychological disturbances in the two groups differ significantly. Exchange transfusion is recommended in healthy full-term infants without iso-immunization as a measure to prevent the serum bilirubin level from exceeding 25 mg/100 ml.

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DEVELOPMENT OF PULMONARY FUNCTION IN LATE GESTATION

I. *The Functional Residual Capacity of the Lung in Premature Children*

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The functional residual capacity (FRC), i.e., the volume remaining in the lung at the end of a normal expiration is, in general, a good index of lung development. FRC has been determined in children and in full-term newborn infants by numerous authors using different techniques. The growth pattern of this and other subdivisions of the lung volume throughout childhood has been recently evaluated in some detail by De Muth et al. (8), and by Polgar & Promadhat (18)

Data of similar measurements (thoracic gas volume (TGV) rather than true FRC) on larger groups of premature infants have been reported by Chu et al. (6), Burnard et al. (5), and by Thibeault et al. (21), using the plethysmographic method of DuBois et al. (9), as modified by Klaus and associates (13). None of these data, however had been analysed from the viewpoint of lung development.

The purpose of the study partially reported in this paper was to investigate the functional development of the lung from the earliest gestational age when viable infants are born, to term, by relating the measured values with age as well as with the attributes of body growth (weight and length). Post mortem studies by others on lung growth and structural development in intra-uterine life were also consulted for comparison with the physiological measurements.

The study also included various measurements of mechanics of breathing (lung compliance and airway resistance) which will be reported separately

This work was presented in part at the 42nd Annual Meeting of the Society for Pediatric Research, Washington DC, 1972. George Polgar was visiting associate Professor on sabbatical leave from the Department of Pediatrics and Physiology School of Medicine, University of Pennsylvania, Philadelphia 19104.

MATERIAL

Among 31 infants studied, 19 were born prematurely with a gestation age of 25 to 36 weeks. Their weights ranged from 680 g to 2300 g. There were two sets of twins in this group. One twin and three singletons were small-for-date. Of the 12 infants born near or at full term, 8 were normally developed for gestational age. Two were twins with low birth weight (1560–2100 g) and two singletons were small-for date secondary to fetal malnutrition. Gestational age could be estimated on the basis of an accurate obstetric history in 12 infants of the premature group whose mothers were followed from the beginning of their pregnancy by the obstetric outpatient service.

In the other newborns, for whom available data were less accurate, clinical assessment of gestational age was determined using the score of Dubowitz and associates (10). In small-for-date infants the Portland fetal growth curves (2) were used for estimation of fetal malnutrition.

In 25 infants the first FRC determinations were made between 10 hours and 8 days after birth, and in 6 infants between 11 and 22 days of age. For the latter 6 post-menstrual age (gestational age plus age at the time of investigation) rather than true gestational age was used. Measurements were repeated 1 to 4 times in 17 infants.

Except for one case with occasional apneic spells, all infants were clinically free of respiratory difficulties, not requiring other than routine care usually

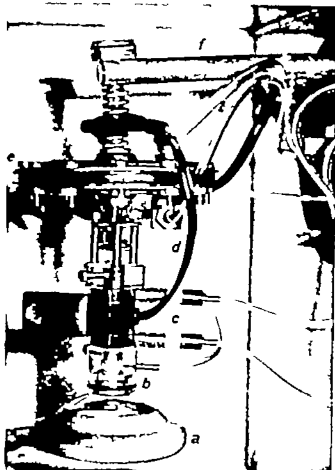


Fig 1 Mask flowmeter-airway shutter system. (*a*) face mask, (*b*) connecting ring with flowmeter (*c*) Fleisch pneumotachograph, (*d*) cable for heating element of flowmeter (*e*) pneumatic airway shutter (*f*) manipulator rod.

given to premature newborns. The smallest baby (680 g), who was symptom free at 1 day of age when studied, died 4 days later with signs of brain hemorrhage confirmed by autopsy

METHODS

FRC was measured by the whole body plethysmographic method of Dubois et al. (9) as adapted to newborn infants by Polgar (16) and further modified for measurements of small prematures by Polgar & Lacourt (19). Briefly a plastic plethysmograph with a volume of 47 liters was used. The mask flowmeter airway shutter system could be manipulated from the outside (Fig 1). Pressure changes in the plethysmograph and at the mouth during respiratory efforts against a closed airway were plotted on a cathode ray oscilloscope screen. In order to measure FRC, the airway shutter was closed at the end of a normal expiration indicated by the plethysmographic pressure tracing The slopes of the plots were determined and FRC calculated according to the calibration factors. A correction was made for the volume of the mask flowmeter tubings and pressure transducer Each value was obtained by calculating the average of 5 to 12 separate measurements.

It was noted that even the smallest prematures continued to make respiratory efforts against the closed shutter so that it took only a few seconds to make a measurement. The complete study of pulmonary mechanics took 15 to 20 minutes for each baby

The plethysmograph was heated only by the baby's own body temperature and by the small heating element in the flowmeter Babies who were kept in open bassinets were put in the plethysmograph in their normal clothing, while those kept in incubators were wrapped in sheets of sterile cotton and blankets. The body temperature of all babies stayed constant during the procedure. No apparent discomfort was caused by and no harmful effect was observed during and after these measurements.

RESULTS

The 57 FRC measurements obtained from the 31 infants studied are summarized in Table 1 FRC is also expressed per kg of body weight, per cm of body length and per week of gestational age The values of FRC ranged from 10 ml to 88 ml in the premature infant group and from 63 ml to 123 ml in the term infants. Table 2 shows the average ratios of FRC to weight, length and gestational age for both groups. The latter two are significantly higher in the mature group but FRC/weight is not significantly different between premature and term infants.

Lung volume and body weight

Figure 2 shows that for babies weighing less than 1 200 g the linear relationship is steeper than for the heavier ones. The regression coefficient of the former is three times larger (0 0814 versus 0 0261) Correlations are good in both groups.

Lung volume and body length

In Figure 3 only the first measurements of FRC are plotted against length since it would have been impossible to accurately measure small increments of length between repeated measurements. The curvilinear relationship is significant at the 5% level and the slope is steeper at both extremes of the curve more so for the longer infants.

Table 1 *Functional residual capacity in premature and term infants*

	Gestational age (weeks)	Age (days)	Weight (g)	Length (cm)	FRC (ml)	FRC/week of gestational age (ml/week)	FRC/kg of body weight (ml/kg)	FRC/cm of body length (ml/cm)
Premature infants								
Ga.	25	1	680	29	10	0.4	14 7	0.34
Pl.	28	1	900	33	20	0.7	22.2	0 6
		5	850		17.5		20.3	
		18	1 030		30		29 1	
		31	1 300		38.6		29.6	
		72	2 650		83		31.3	
Vi.	28	6	970	37	33	1 17	34	0.9
Ka.	30	4	880	34	23	0.76	26.1	0 67
		13	970		34		35	
Ca.	30	5	1 050	37	44	1 46	42	1 19
Du.	30	7	1 130	41	47	1.56	41.5	1 14
		15	1 250		49		39.2	
Pr	31	3	1 100	39	36	1 13	32.5	0.92
Ba	33	7	1 400	44	48	1 46	34.2	1.09
		16	1 600		50		35	
		28	1 900		74		38.9	
Ra	35	7	1 700	44	55	1.56	32.3	
		18	1 960		56		28.5	
		21	2 030		55		28	1.25
Ma.	35	6	1 660	44	55	1.56	33 1	
Pa. I	35	7	2 300	47	53	1.51	23	1.12
		11	2 400		62		25.7	
		16	2 580		67		25 9	
Pa. II	35	7	2 250	45	54	1.53	24	
		20	2 500		72		28.8	
Sh.	35	18	1 500	39	50	1 4	33.3	1.28
		25	1 580		44		27.8	
		32	1 960		53		27	
Ar	35	14	1 800	44	88	2.5	48 8	2
Bo.	35	22	1 800	42	46	1.3	25.5	1 1
Du.	35	31	2 030	45	45	1.3	22.5	1
		42	2 400		67		27.9	
Sa.	36	2	1 700	44	61	1 7	35.8	1 4
		11	1 950		55		28.2	
		27	2 320		68		29.3	
Ra. I	36	15	1 850	42	55	1.53	29 7	1 4
		22	2 020		50		25	
Ra. II	36	15	1 690	40	53	1 47	31 1	1.32
		22	1 850		63		34	
Term infants								
Bo.	37	1	2 000	45	70	1.88	35	1.5
Wu.	38	3	2 570	48	70	1.84	26.9	1 45
Va.	38	7	2 560	49	68	1 79	26.8	1 4
Gi. I	40	3	1 560	43	63	1.57	39.3	1 46
		5	1 520		65		42.7	
Gi. II	40	5	2 100	46	67	1.67	31.9	1 45
Mo.	40	4	2 180	45	80	2	36.7	1 77
		7	2 180		80		36.7	
		8	2 200		77		35	
		18	2 350		97		41.2	
Ca.	40	1	2 900	48	63	1.57	21 7	1.3
		2	2 880		65		22.6	
Mo.	40	1	3 460	50	93	2.32	26.8	1.86
Pi.	40	1	3 200	50	90	2.25	26.1	1.8
		3	2 970		74		24.9	
Am.	41	6	3 130	52	110	2.68	35.1	2.1
An.	41	6	3 600	51	120	2.9	33.3	2.3
Ba.	42	2	3 350	52	123	2.9	39 7	2.3

Table 2. *Average ratio of functional residual capacity over body weight length and weeks of gestation*

	Infants	Functional residual capacity/kg of body weight (ml/kg)	Functional residual capacity/cm of body length (ml/cm)	Functional residual capacity/week of gestation (ml/week)
	n	$\bar{x}$ S D	$\bar{x}$ S D	$\bar{x}$ S.D
Premature infants	19	30.4 ± 6.63 (39[a])	1 12 ± 0.36 (19)	1.37 ± 0 44 (19)
Term infants	12	32.4 ± 6.4 (18)	1 72 ± 0.35 (12)	2.11 ± 0 49 (12)
Significance of difference		Not significant	$p < 0\ 001$	$p < 0.001$

[a] Number of measurements.

Lung volume and gestational age

The relationship (Fig. 4) is very similar to the previous one The steepest part of the curve is after 36 weeks of gestation Extrapolation over a very short range to zero FRC on the time axis would result in an intercept just below 24 weeks gestation

DISCUSSION

In older children and in adults lung volume is best correlated with height (8–18) In premature babies this may also be so However because of the difficulties involved in accurately measuring length at this age the relationship of lung volume with the easier accessible data on body weight must also be considered. Gestational age is a particularly important parameter in evaluating development of premature infants. However this parameter can only be used for correlation with pulmonary function if proper means of estimation, such as precise obstetrical history and physical and neurological data, are available.

In evaluating and interpreting our measurements based on the above three attributes of growth we concentrated on their significance from the viewpoint of fetal development. We have measured lung volumes that are fairly evenly distributed within the range of viable prematures, therefore we could compare this physiological development in late gestation

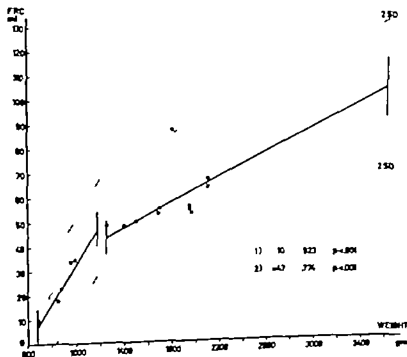


Fig 2 The relationship of 57 measurements of FRC with body weight in 31 infants expressed by two linear regressions. 1) Line on the left for infants weighing less than 1 200 g: FRC = 48 42 + (0.0814 ± 0.0239) × weight. 2) Line on the right for infants weighing more than 1 200 g: FRC = 10.9 + (0 0261 ± 0 0065) × weight. The number of points (n), the coefficients of correlation (r) and probabilities (p) are given separately for both relations.

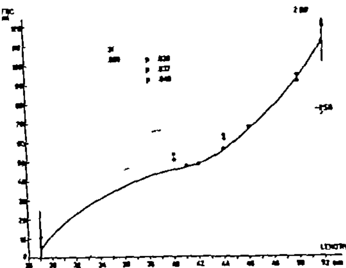


Fig. 3. Relationship of the first measurements of FRC with body length (cm) in 31 infants. FRC = $(1.857 \pm 1.596) \times 10^{-4} \times \text{length}^3 - (2.178 \pm 1.983) \times \text{length}^2 + (8.728 \pm 8.097) \times 10 \times \text{length} - 1149.29$. Coefficient of correlation (r) and probabilities (p) for the 3 sections of the curve are given.

with different functional and anatomical variables examined by others.

Some of the peculiar patterns shown for the relationship of FRC with weight, length and age in Figs. 2, 3 and 4 can be explained by considering the patterns of growth velocity for weight and length in late gestation. Velocity curves plotted from data by Lubchenko et al. (14, 15) do not follow a continuous pattern (Fig. 5). Their lowest values are in the age range of less than 30 weeks, and there is another low phase after 36 weeks of gestation. In the first period of low growth rate, weight increases even more slowly than length.

The FRC to gestational age relationship has one steeper part in the range of the youngest premature infants, and another one in the range after 36 weeks of gestation. The initial acceleration of FRC development taking place when both weight and length velocities are low is in accordance with the more rapid rate of growth of lung volume relative to weight, and less so in length, as seen in the initial parts of the curves in Figs. 2 and 3 respectively.

It should be noted that the point of intersection between the two slopes of the FRC/weight relationship corresponds to 1 200 g fetal weight and a gestational age of 30 weeks at which, (Fig. 5) the weight velocity curve and the length velocity curve cross over. The

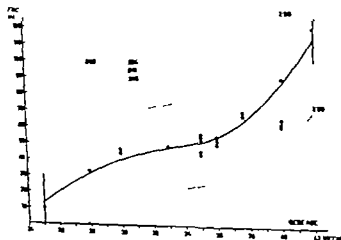


Fig. 4. Relationship of the first measurements of FRC with gestational age (weeks) in 31 infants. FRC = $(5.898 \pm 5.267) \times 10^{-4}\ \text{weeks}^3 - (5.753 \pm 5.359)\ \text{weeks}^2 + (1.890 \pm 1.797) \times 10^2 \times \text{weeks} - 2047.43$. Coefficient of correlation (r) and probabilities (p) for the 3 sections of the curve are shown.

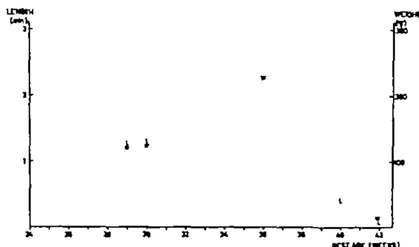


Fig 5 Velocity curves of growth in weight (*W*) and in length (*L*) during gestation plotted from data obtained by Lubchenko et al. (*Pediatrics 32* 793 1963 and *37* 403 1966).

flatter part of the FRC/weight relationship corresponding with weights larger than 1 200 g indicates that lung volume is growing almost in proportion to weight

The accentuated steepness of the last portion of the FRC/length curve can only partly be explained by the low length growth velocity toward the end of gestation. In fact the acceleration of FRC development versus age found after 36 weeks of gestation (Fig 4) proves that another factor is also present.

The independent acceleration of lung volume in late gestation had also been suggested by Emery & Mithal (11) who found a sudden increase in the number of alveoli per terminal lung unit after 36 weeks gestation (Fig 6)

The significantly larger FRC/length and FRC/gestational age ratio shown in term infants as compared with premature babies (Table 2) further supports the existence of an increased rate of FRC development toward the end of gestation A similar tendency can be found in the results of Burnard et al. (5) who reported an FRC/length ratio of 1.38 ml/cm for normal premature infants studied during the first 3 weeks of life and 1 6 ml/cm for term babies

The measurements of Thibeault et al. (21) made on material very similar to ours, reveal a mean FRC/length ratio of 1 03 ml/cm, almost identical to our findings for premature infants. However they show no tendency toward a sudden increase of this ratio (1 09 ml/cm) in the longest or term babies. The latter discrepancy may be related to the fact that the average absolute lung volume for full term babies in the quoted study is smaller than noted in the results of several authors, including those reported here On the basis of the majority of opinion, we do believe that

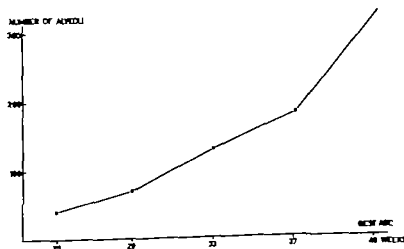


Fig 6 Estimated number of alveoli per terminal lung unit plotted from data by Emery & Mithal (*Arch Dis Childh 35* 544 1960).

he discontinuous growth in lung volume at his stage of development is, as reported in his paper indeed a true phenomenon.

Mean FRC/weight ratios for term babies reported by previous workers (1 3 6, 7 12, 13 17) or calculated from their data range from 21.5 to 38 ml/kg with an average of 29.2 ± 6 1 ml/kg. Our results for the same group of infants are very similar with an average value of 32.4 ± 6.4 ml/kg Published data for this same ratio in premature infants range from 20.5 to 47.8 ml/kg (5 6, 20, 21) with an average of 31 1 ± 11 8 ml/kg, which is also very similar to the one in our group (30.4 ± 6.63 ml/kg). Like Burnard et al. (5), but in disagreement with Chu et al. (6) and Thibeault et al. (21) we found no significant difference between the full term and premature group. Recently using the helium dilution technique in 4 prematures weighing about 2 100 g. Ronchetti and co-workers reported that the FRC/weight ratio for these infants was smaller than in term babies (20). It is too early to decide (in the light of the few comparative studies available at the present time) which of these patterns is the more realistic one. However the fact that in healthy adults this ratio is the same as the one we found for both premature and term infants seems to indicate the existence of a constant ratio throughout life.

The extrapolated intercept to zero FRC on the time axis in Fig. 4 is of special interest. Although such extrapolation must be interpreted with extreme caution, in this case the distance of extrapolation is so small (from 10 ml to 0 ml) that it seems justifiable This intercept at 24 weeks gestation corresponds precisely with the period when anatomical alveolization of the lung has been reported to begin (4) We have therefore confirmed by physiological measurements the time in gestation after which viable babies can be expected, as far as the development of lung volume is concerned. There are, of course *other factors* determining the readiness of the respiratory system for functioning.

In variance with Thibeault et al. (21) findings, but in agreement with Chu et al. (6) and Klaus et al. (13) our repeated measurements (Table 1) suggest that the lung volume as established very soon after birth does not decrease during the first week of life. Actually several measurements of FRC postnatally followed the *same growth pattern as they* would have by *intrauterine* development.

SUMMARY

Functional residual capacity has been measured in premature and term infants soon after birth and repeatedly thereafter Good correlation with body weight, length and gestational age have been found. However the known relationships between the development of lung volume and attributes of body growth in older individuals are only partially valid during late gestation. Some peculiar growth patterns observed in this age could be explained by disproportionate variations in the rate of growth of body length and weight on the one hand and of lung volume on the other

By extrapolation over a very short *range* it was possible to verify the post mortem estimates of the gestational age at which alveolization occurs.

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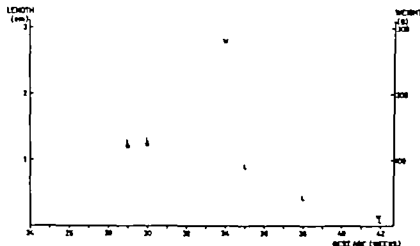


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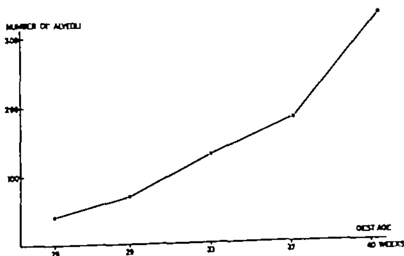


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THE "BLUE" METER, A PHOTOMETER DESIGNED TO MEASURE LIGHT EMISSION DURING PHOTOTHERAPY OF HYPERBILIRUBINEMIA

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The widespread application of phototherapy in the treatment of hyperbilirubinemia of the newborn has raised the problem of a suitable meter for a uniform measurement of the efficiency of the different fluorescent lamps in use. Furthermore, dependent on the phosphors employed and the operational circumstances, especially the temperature, the emission of light energy from these lamps is time-limited, even if the light spectrum changes but little (4).

The spectral distribution curve of the photo-oxidation of bilirubin in serum was first recorded by Cremer et al. (1), who showed that this process had its optimum at 450 nm, which is the absorption maximum of bilirubin in serum. Later this action spectrum of bilirubin photo-oxidation has been revised by Glauser et al. (3) who have demonstrated a spectrum with 4 peaks. The largest amount of bilirubin oxidation occurs at 450 nm with a half-bandwidth of slightly less than 20 nm. There are two relatively less efficient wavelengths at 410 and 370 mm, of which the one at 370 nm probably is of little importance as the Plexiglas shields in front of the lamps screen out all but 2–3% of the beam intensity below 390 nm. Recently the action spectrum has been modified by Lanning et al. (5), who have corrected the curve for light attenuation in the skin by measuring light reflectance in the skin (Fig. 1)

Footcandles and Lumen are photometric units adapted to the visual sensation of the human eye which is quite different from the sensitivity curve of the bilirubin photo-oxidative system, that has its peak in the blue part of the spectrum. Watt, and Watt/m² are radiometric units expressing radiant flux and irradiance, respectively When measuring light energy emitted by lamps used in phototherapy spectral selective or weighting instruments are required.

On the reported experimental background a photometer has been developed by the Danish Illuminating Engineering Laboratory in order to measure the efficiency of fluorescent lamps used in the treatment of hyperbilirubinemia. As the meter has maximal sensitivity in the blue part of the spectrum it is called The "Blue" Meter It is a spectral weighting type instrument, which employs a special broad-band weighting curve ($B(\gamma)$)

Theory of operation

The overall spectral response of The "Blue" Meter is matched to the action spectrum of the photo-oxidation of bilirubin corrected for skin attenuation (5). In Fig. 1 are shown the required and the actual

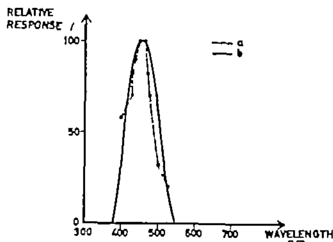


Fig 1 The spectral response of The "Blue" Meter (*a*) matched to the action spectrum of bilirubin photo-oxidation corrected for skin attenuation (5) (*b*).

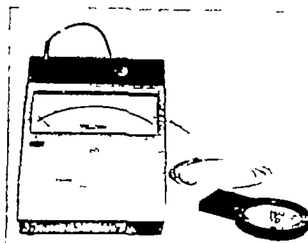


Fig 2. The "Blue" Meter See description.

relative spectral response of the meter As this response differs from spectral responses of instruments used in photometry and radiometry The "Blue Meter is calibrated in new units called Watts/m (*B*) where (*B*) indicates the special spectral weighting of the blue filter The definition of the unit Watt/m follows from the equation, $E_B = \int_{\lambda_1}^{\lambda_2} E\lambda\, B(\lambda)\, d\lambda$ where E_B is the measured quantity in Watt/m² (*B*) $E\lambda = dE/d\lambda$ is the spectral concentration of irradiance in Watt/m per nm $B(\lambda)$ is the actual relative response and λ is the wavelength in nm The integration limits λ_1 and λ_2 for points at which the $B(\lambda)$ curve falls to about 1% of the maximal value are 380 and 540 nm respectively

Description

The Blue Meter consists of two units, the removable optical head and the measuring instrument connected by a cable (Fig. 2). The optical head is an EEL standard (uncorrected) barrier layer selenium cell with a diameter of the active area of 62 mm fitted with a Kodak light blue wratten filter type 47 A. The measuring instrument is a modified H. & B Illumination meter type EBLX3 (internal resistance 2180 Ω FSD current 12 μA), provided with a six position range switch and a coaxial socket

Specifications

The measuring instrument is a moving coil instrument with torsional suspension, provided with a mirrored scale. Scale length 115 mm. Scale division 0–10 and 0–3

There are 5 measuring ranges. 0.3 1 3 10 and 30 Watt/m² (B) selectable by a switch provided also with an OFF" position.

The overall relative spectral response, peaking at 445 ± 5 nm is shown in Fig 1

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DISCUSSION

The reported "Blue Meter is based on the present knowledge of the action spectrum of bilirubin photo-oxidation *in vitro* It does not take into account, that in the *in vivo* system this spectrum may be modified by complex and unknown factors, which may differ in the individual patient. For instance an inverse relationship has been demonstrated between the serum albumin concentration and the photo-oxidation rate (2) This and other variables may explain different responses to phototherapy in an otherwise uniform group of hyperbilirubinemic patients.

SUMMARY

In order to measure the efficiency of fluorescent lamps used in phototherapy a new photometer has been devised. The spectral response of the meter is matched to the action spectrum of bilirubin photo-oxidation corrected for skin attenuation. The theory of operation and a technical description of the instrument is given

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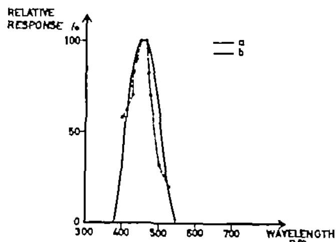


Fig 1 The spectral response of The "Blue" Meter (*a*) matched to the action spectrum of bilirubin photo-oxidation corrected for skin attenuation (5) (*b*).

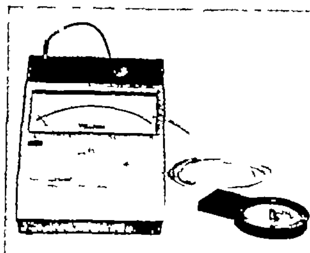


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Table 1 *Percentage of isotope excreted in urine and faeces during 3 days following administration of lithocholic acid-24-¹⁴C either orally or intramuscularly*

Patient	Age (weeks)	Administration	Sampling period (hours after administration)	% of given isotope recovered in	
				Urine	Faeces
C. E.	35	Oral	0–24	11.5	42.5
			24–48	3.1	1.4
			48–72	1.1	0
				15.7	*43.9*
M. A.	13	Intramuscular	0–24	20.2	<0.1
			24–48	15.8	<0.1
			48–72	8.6	<0.1
				44.6	<0.1

teriorated rapidly and she died at 9 months. Autopsy confirmed the absence of the gall bladder and the extrahepatic bile ducts. The liver was hard and finely nodular. Numerous small cavities containing viscous bile were present in its centre. The spleen was enlarged and hard. The stomach contained blood and she had oesophageal varicoses with one suspect mucosal erosion.

M. A., a boy born at term, was the first child, his birth weight and birth length being 2 570 g and 46 cm, respectively. Apart from a common cold which the mother developed 1 week before delivery and which rapidly subsided, her obstetrical history was normal. His father was healthy and the mother subsequently gave birth to a normal child. The patient had bilaterally a supernumerary fifth digit and retained testis on one side. At the age of 2 months jaundice was noticed and he had clay-coloured stools. The liver was enlarged and firm. Since jaundice persisted, explorative laparotomy was performed at 2.5 months of age, which revealed a hypoplastic gall bladder and the absence of the cystic duct, the hepatic ducts and the common bile duct. Thereafter jaundice deepened, total serum bilirubin being about 30 mg/100 ml. His general condition deteriorated and he died at the age of 15 weeks. At autopsy neither the gall bladder nor the extrahepatic bile ducts could be detected. The liver was enlarged, hard and nodular and showed pronounced bile stasis, proliferation of the bile ducts and a large number of inflammatory cells.

METHODS

Lithocholic acid-24-¹⁴C was synthesized according to the method of Bergström et al. (1). Lithocholate-3-sulphate, taurolithocholate 3-sulphate and glycolithocholate-3-sulphate were synthesized according to Palmer & Bolt (16).

The lithocholic acid-24-¹⁴C was administered as sodium salt dissolved in 70% ethanol. Patient CE was given 10 μCi (0.1 mg) orally by a gastric tube and patient MA was given 10 μCi (0.1 mg) intramuscularly. The patients wore urinary bags and paper diapers during the sampling period. If the stools were loose the isotope was extracted from the diapers and faeces together. As some urine may be passed into the diapers the latter were divided into two groups; one including diapers which contained only urine and the other including diapers containing both urine and faeces. Urine, faeces and diapers were extracted as previously described and the isotope was determined by liquid scintillation technique (13).

Urine, 30 ml, was passed through an Amberlite XAD-2 column and the bile acids eluted with methanol (13). An aliquot of the residue was chromatographed on a 4 g column of Sephadex LH 20 using 400 ml of chloroform-methanol (1:1) with 0.01 M potassium chloride as the mobile phase. Fractions of 5 ml were collected. Unconjugated and conjugated bile acids were eluted between 125 and 200 ml effluent. A second fraction of bile acid sulphates was obtained by final elution of the column with methanol. Each fraction was analyzed by thin-layer chromatography (TLC). Radioactive spots were located by autoradiography (13). Labelled conjugates were isolated by preparative TLC. Aliquots of the different fractions were subjected to enzymatic hydrolysis (17) and to solvolysis (14) and thereafter rechromatographed by TLC. An aliquot of the methanol residue was subjected to solvolysis, alkaline hydrolysis and methylation and the final ether extract was analyzed for unconjugated bile acids by TLC and aluminium oxide chromatography followed by TLC and gas-liquid chromatography (GLC) (12, 13).

RESULTS

Excretion rate of isotope

The recovery of the isotope from urine and faeces is summarized in Table 1. After oral administration about half of the amount of isotope was found to be absorbed. After intramuscular injection 44% of the administered isotope was excreted in the urine for 3 days.

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METABOLISM OF LITHOCHOLIC ACID-24-^{14}C IN EXTRAHEPATIC BILIARY ATRESIA

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Infants with extrahepatic biliary atresia excrete large amounts of conjugated bile acids, tri- di and monohydroxy bile acids in the urine (9 13) Most of the tri and dihydroxy bile acids are cholic and chenodeoxycholic acids. The monohydroxy bile acids consist of several different compounds of which 3β-hydroxy 5-cholenoic acid is quantitatively the most important Additional findings in the urine of these infants were small amounts of lithocholic and allolithocholic acids (9)

In adults cholesterol is transformed to the primary bile acids cholic and chenodeoxycholic acids, which are metabolic end products and are excreted in the bile Small amounts of lithocholic acid have also been found in the bile (3) probably formed by 7α-dehydroxylation of chenodeoxycholic acid by the action of microbial enzymes. Investigations of the metabolism of lithocholic acid 24-^{14}C in adults have shown that the latter is transformed to a small extent (2) mainly during the enterohepatic circulation (11) Sharp et al. have isolated lithocholic acid from meconium (18)

In a previous work on bile acid metabolism in extrahepatic biliary atresia, cholic acid and essentially also chenodeoxycholic acid have been shown to be metabolic end products (12). No transformation of the cholic acid-24-^{14}C occurred and of the chenodeoxycholic acid-24-^{14}C, 4–7% was transformed. The nature of the products was not determined.

For further study of the metabolism of monohydroxycholanoic acids in infants with extrahepatic biliary atresia, lithocholic acid-24-^{14}C was given to 2 infants with this disease. The aim was (i) to study whether lithocholic acid is a metabolic end product in these cases and (ii) to study the elimination rate of the isotope

The systematic names of the bile acids referred to by trivial names are as follows: cholic acid, 3α 7α, 12α-trihydroxy 5β-cholanoic acid α-muricholic acid, 3α, 6β 7α-trihydroxy 5β-cholanoic acid β-muricholic acid 3α, 6β 7β-trihydroxy-5β-cholanoic acid hyocholic acid, 3α, 6α, 7α-trihydroxy 5β-cholanoic acid, chenodeoxycholic acid, 3α, 7α-dihydroxy 5β-cholanoic acid hyodeoxycholic acid 3α, 6α-dihydroxy 5β-cholanoic acid ursodeoxycholic acid, 3α 7β-dihydroxy 5β-cholanoic acid, lithocholic acid, 3α-monohydroxy 5β-cholanoic acid isolithocholic acid, 3β-monohydroxy-5β-cholanoic acid, allolithocholic acid, 3α monohydroxy 5α-cholanoic acid

CASE REPORTS

C E a girl, born at term, was the second child, her birth weight and birth length being 2 800 g and 50 cm, respectively She had 2 healthy siblings. Her parents were healthy and her mother's obstetrical history was normal. Jaundice persisted and she had clay-coloured stools since birth. At the age of 7 weeks she was admitted to hospital because of slow weight gain, hepatomegaly and jaundice. At the age of 3 months laparotomy was performed, which revealed the absence of the gall bladder and the extrahepatic bile ducts. When she was 8 months old she developed pronounced ascites and was treated by laparocentesis on several occasions. There was no pruritus. Her condition de-

ounds. The major compound showed the same mobility as lithocholic acid and that of the other one was slower indicating the presence of a double bond.

Fraction (C) contained mainly one labelled compound at TLC with a mobility between the mobilities of cholic and chenodeoxycholic acids. The mobility of the compound corresponded to that of hyodeoxycholic acid but not to those of α- or β-muricholic acids, hyocholic acid or ursodeoxycholic acid. Analysis by GLC indicated that it was not hyodeoxycholic acid. The nature of this compound is unknown. Trace amounts of a more hydrophobic compound than the major one were also found. The nature of this latter compound is likewise unknown.

DISCUSSION

Animals metabolize lithocholic acid in different ways. In the rat several metabolites are formed. chenodeoxycholic, 3α, 6β-dihydroxy-5β-cholanoic, α-muricholic and β-muricholic acids (10, 19). In the rabbit no transformation of lithocholic acid occurs (6). In the hog hyodeoxycholic acid is the major metabolite (7). In the dog the only metabolite formed from lithocholic acid is chenodeoxycholic acid (8).

In man lithocholic acid has not been found to be hydroxylated. Lithocholic acid-24-^{14}C given to adults is slowly transformed to isolithocholic acid and 3-keto-5β-cholanoic acid (11). These transformations occur during the enterohepatic circulation and might be caused by the action of microbial enzymes.

The present investigation showed that lithocholic acid-24-^{14}C is transformed to a small extent to more polar compounds. This is in agreement with results obtained in adults (2). The major polar metabolite was not identical with cholic acid, chenodeoxycholic acid, α- or β-muricholic acids, hyocholic, hyodeoxycholic acid or ursodeoxycholic acid. The major urinary labelled compounds consisted of monohydroxycholanoic acids which essentially were unmetabolized lithocholic acid. Small amounts of an unsaturated monohydroxycholanoic acid were also detected, which finding is in agreement with previous investigations in adults (11).

In animals 3β-hydroxy 5-cholenoic acid has been shown to be metabolized to lithocholic acid before further transformation (10). Infants with extrahepatic biliary atresia excrete large amounts of 3β-hydroxy-5-cholenoic acid but only trace amounts of lithocholic acid (9). The relative slow excretion rate of mainly unchanged lithocholic acid observed in the present investigation indicates that very small amounts of lithocholic acid are formed in infants with extrahepatic biliary atresia.

Lithocholic acid has repeatedly been shown to be hepatotoxic due to its cholestatic effect (5). The cholestatic effect of the corresponding taurine and glycine conjugates is weaker and that of the sulphate esters of these conjugates has been shown to be still weaker (4). In the present investigation lithocholic acid was found to be almost entirely excreted in the form of the tauro- and glycolithocholate-sulphates in the urine of infants with extrahepatic biliary atresia. By sulphation the bile acid conjugates become more soluble in water. This facilitates the urinary excretion of lithocholic acid (15). The results of the present investigation supports Palmer's hypothesis (14) that sulphation of lithocholic acid may serve as an important physiological method of protecting the organism from the toxic effect of this endogenous compound.

The high degree of sulphation of lithocholic acid conjugates observed in the present investigation and the low quantitative excretion of lithocholic acid previously observed (9) suggest that lithocholic acid is no important factor in the rapid progression of cirrhosis observed in extrahepatic biliary atresia.

SUMMARY

Lithocholic acid-24-^{14}C was given to 2 infants with extrahepatic biliary atresia. After oral administration about half of the amount of

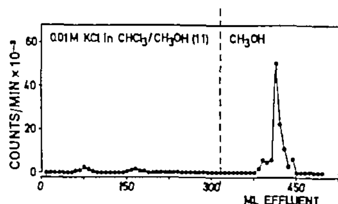


Fig 1 Chromatogram on Sephadex LH 20 of methanol eluate of urine after percolation through Amberlite XAD-2 column (patient MA) Moving phase: 0 01 M KCl in chloroform/methanol (1 1) followed by methanol.

Separation and identification of labelled conjugates

At chromatography on Sephadex LH 20 lithocholic taurolithocholic and glycolithocholic acids were recovered in the fractions 125–200 ml effluent The corresponding sulphate esters were not eluted with 400 ml moving phase chloroform–methanol but were eluted with methanol Aliquots of urine from patients CE and MA were chromatographed on Sephadex LH 20 The results showed that small amounts of the isotope were eluted with chloroform–methanol whereas 87 and 90% respectively were recovered in the final methanol eluate (Fig 1) TLC of the chloroform–methanol fractions revealed several labelled conjugates but no unconjugated bile acids TLC of the methanol fractions revealed two labelled compounds with the mobilities of glycolithocholate-3-sulphate and taurolithocholate 3-sulphate but not with the mobility of lithocholate 3-sulphate. After solvolysis the mobilities of the labelled compounds were identical with those of taurolithocholic and glycolithocholic acids. After enzymatic hydrolysis they were identical with that of lithocholate 3-sulphate.

Separation of labelled bile acids after solvolysis and hydrolysis

The residues of the final ether extracts of the urine from the 2 patients were methylated and subjected to column chromatography on aluminium oxide (Fig. 2) Labelled compounds were eluted in the first fractions (A) and at the places where monohydroxy (B) and trihydroxycholanoic (C) acids were eluted. Small amounts of isotope were eluted just before and after (B)

Fraction (A) yielded labelled lithocholic acid by TLC after mild hydrolysis, thus indicating that a derivative of lithocholic acid other than the methyl ester had been formed during the procedures of extraction and purification.

Fraction (B) was subjected to TLC and found to have the mobility of lithocholic acid methyl ester No labelled compound was present as isolithocholic acid TLC on plates containing silver nitrate revealed two labelled com-

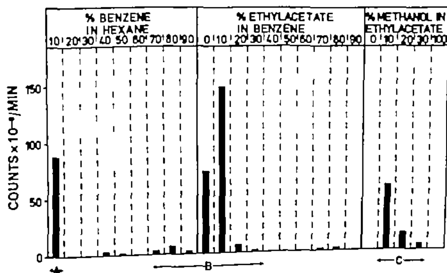


Fig 2 Alumina fractionation of the methylated ether extract of urine from patient *MA* 2 days after administration of lithocholic acid-24-^{14}C. Columns: 8.5 g of aluminium oxide.

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URINARY BILE ACID CONJUGATES IN EXTRAHEPATIC BILIARY ATRESIA

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Normal infants excrete most of the primary bile acids, cholic and chenodeoxycholic acids, into the intestine conjugated with taurine (3). In extrahepatic biliary atresia and intrahepatic cholestasis of infancy bile acid excretion is disturbed and this results in virtually all of the bile acids being excreted in the urine where they appear in conjugated form (10, 11, 14). In a previous investigation of infants with intrahepatic cholestasis, apart from labelled glycocholic and taurocholic acids, several other labelled conjugates were isolated from the urine after the administration of cholic acid-24-^{14}C (14). The possibility that small quantities of bile acids are involved in an enterohepatic circulation cannot be excluded in these infants with normal bile ducts. The bile acid conjugates were therefore investigated in 5 infants with extrahepatic biliary atresia, since any effect of the microbial flora on the metabolism of bile acid conjugates could be reasonably excluded in these infants. After the administration of cholic acid-24-^{14}C, chenodeoxycholic acid-24-^{14}C and the [1-^{14}C]-glycine labelled conjugates of cholic and chenodeoxycholic acids, the labelled bile acid conjugates excreted in the urine were separated and their nature studied. The results of these studies are reported in this paper.

The systematic names of the bile acids referred to by trivial names are as follows: *cholic acid*, 3α, 7α, 12α-trihydroxy-5β-cholanoic acid, chenodeoxycholic acid, 3α, 7α-dihydroxy 5β-cholanoic acid; lithocholic acid, 3α-monohydroxy 5β-cholanoic acid.

CASE MATERIAL

Five patients with extrahepatic biliary atresia were studied. The histories of 4 of the patients (HL, KJ, CE and AM) were reported previously (10). The history of the fifth patient, JS is given below.

JS, a boy, was the second child, birth weight and birth length being 4 760 g and 55 cm respectively. His parents were healthy and so was his elder sister. His mother's pregnancy was uneventful. He developed hyperbilirubinaemia in the neonatal period, maximal serum bilirubin concentration being 18.1 mg/100 ml on the 3rd day of life. He was jaundiced since birth and was admitted to hospital at the age of 2 months because of persisting jaundice, hepatosplenomegaly and clay-coloured stools. A liver biopsy showed pronounced canaliculare bile stasis. At the age of 5 months an explorative laparotomy was performed; neither the extrahepatic bile ducts nor the gall bladder could be detected. A liver biopsy showed excessive bile stasis, proliferation of the bile ductules and absence of the bile ducts. Thereafter he developed ascites but could be kept in a reasonable condition with conservative therapy. There were several episodes of fever. X-ray revealed a slightly enlarged heart. He died at the age of 11 months after some days of rapid progression of the ascites. At autopsy the liver was found to be hard, green-coloured and finely nodular. Microscopy revealed advanced cirrhosis and bile stasis. Only connective tissue with some mononuclear cells were found at the sites of the hepatic ducts. *Fibrotic strings* were seen at the sites of the gall bladder and the common bile ducts. The left cardiac ventricle was hypertrophic but the valves were normal.

isotope was found to be adsorbed After parenteral administration about half of the amount of isotope was excreted in the urine for 3 days. Lithocholic acid was shown to be transformed to a small extent to more polar compounds. These metabolites were not cholic chenodeoxycholic, α or β-muricholic, hyocholic hyodeoxycholic or ursodeoxycholic acids. Most of the lithocholic acid was excreted as the sulphate esters of tauro- and glycolithocholic acid. No sulphate ester of lithocholic or free lithocholic acid was excreted

ACKNOWLEDGEMENT

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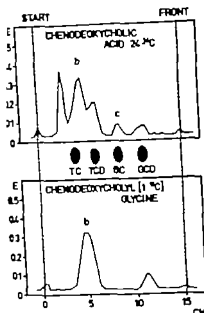


Fig. 1 TLC-separation of urinary labelled metabolites after administration of chenodeoxycholic acid-24-^{14}C (patient AM) and chenodeoxycholyl[1 ^{14}C]glycine (patient JS). Solvent system II. Reference substances: taurocholic acid (TC), glycocholic acid (GC), taurochenodeoxycholic acid (TCD) and glycochenodeoxy cholic acid (GCD). Crosshatching represents spots detected by spraying with phosphomolybdic acid. Densitometric recording of the autoradiograph is given.

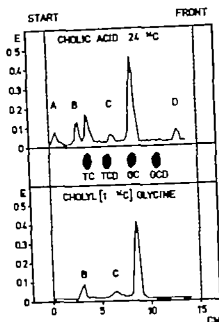


Fig. 2 TLC-separation of urinary labelled metabolites after administration of cholic acid-24-^{14}C (patient HL) and cholyl[1 ^{14}C]glycine (patient HL). For explanation, cf. *fig. 1*

part). Most of the isotope was found in two broad peaks, *a* and *b* and smaller amounts in peak *c*

Chenodeoxycholyl[1 ^{14}]glycine Only small amounts of the labelled compound showed the TLC mobility of unchanged glycochenodeoxycholic acid. Most of the isotope consisted of a compound with the mobility of compound *b* (Fig. 1 lower part).

Cholic acid 24-^{14}C A representative chromatogram is shown in Fig. 2 (upper part). In all 4 patients investigated, most of the labelled compounds were found at the positions of taurocholic and glycocholic acids. Apart from these positions labelled compounds were consistently found in four other regions, A–D

Cholyl[1 ^{14}C]glycine Most of the labelled compound appeared as glycocholic acid but labelled compounds also appeared at the positions B and C (Fig. 2, lower part).

Chromatography on columns of Sephadex LH 20

Chenodeoxycholic acid-24-^{14}C and chenodeoxycholyl[1 ^{14}C]glycine. Most of the labelled compounds found after the administration of chenodeoxycholic acid-24-^{14}C were recovered in the methanol eluate (IV) (Fig. 3). The other

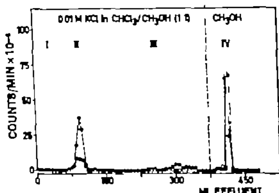


Fig. 3 Column chromatography of urinary labelled metabolites on Sephadex LH 20. Urine extract from patient AM after administration of chenodeoxycholic acid-24-^{14}C (○—○) and from patient JS after administration of chenodeoxycholyl[1 ^{14}C]glycine (●—●).

Table 1 *Age amount of given isotope and percentage excreted isotope in the patients*

	Cholic acid-24-^{14}C				Chenodeoxycholic acid-24-^{14}C		Cholyl [1 ^{14}C] glycine		Chenodeoxycholyl [1 ^{14}C] glycine
Patient	H. L.	K. J	J S.	C. E.	A. M	J S.	H. L.	K. J	J S.
Age at the examination, weeks	8	8	19	11	13	28	20	14	40
Amount of labelled bile acid,									
μCi	7.5	7.5	7.5	7.5	10	10	10	10	10
mg	0 05	0 05	0 05	0 05	1	1	1	1	1
Route	im*	im	im	im	im	oral	im	im	im
Excreted isotope during 4 days, % of given amount	38	88	68	62	17	17	68	33	40

* Intramuscular

MATERIAL AND METHODS

Labelled compounds. Cholic acid-24 ^{14}C was obtained from New England Nuclear Corp., Boston, Mass. Chenodeoxycholic acid 24 ^{14}C was synthesized and purified according to Bergström et al (1) Cholyl [1 ^{14}C]glycine and chenodeoxycholyl[1 ^{14}C]glycine were synthesized and purified as described earlier (8)

Bile acid sulphates. The sulphate esters of chenodeoxycholic acid and its taurine and glycine conjugates were prepared with sulphur-trioxide as described by Palmer & Bolt (17) The reaction mixture was allowed to stand at room temperature for 1 day The butanol extract was separated by column chromatography on Sephadex LH 20 (see below) into mono- and disulphate fractions.

Experimental design Labelled bile acids were autoclaved and dissolved in saline (1.0 ml) before intramuscular injection or oral administration by intubation Urine and faeces were collected daily during the following 4 days (11). The amounts of labelled bile acids given and the ages of the infants investigated are listed in Table 1

Chromatographic separation of labelled urinary bile acids

Urine (30 ml) was percolated through Amberlite XAD-2 (15 gram) and the bile acids eluted with methanol. The residue was chromatographed on columns of Sephadex LH 20 and subjected to thin-layer chromatography (TLC) Aliquots of the different chromatographic fractions were subjected to solvolysis, alkaline hydrolysis or enzymatic hydrolysis (18) and rechromatographed on TLC (For details see 10 11).

Chromatographic technique

Chromatography was performed on 24 g columns of Sephadex LH 20 using an elution with 400 ml of chloroform-methanol 1 1 (v/v) with 0.01 M potassium chloride. Thereafter an elution with 200 ml methanol was used to elute the sulphates. The mono- and disulphates of chenodeoxycholate and glycochenodeoxycholate were separated on columns of Sephadex LH 20 by increasing the concentration of methanol in chloroform in the mobile phase. Monosulphates were eluted with chloroform/methanol 3 7 whereas disulphates appeared in chloroform/methanol 1 9

Thin layer chromatography (TLC) of conjugated urinary bile acids and of the mono- and disulphates of chenodeoxycholate and their corresponding glycine and taurine conjugates was performed with the following solvent systems. I n-propanol/propionic acid/isoamylacetate/water 10 15 20 5 II n-butanol/acetic-acid/water 50 5 5 III n-butanol/0.01 M tris-buffer/propionic acid 50 9.25 0 75 (17) To isolate different labelled compounds the silica gel with the bile acid spots concerned were removed and extracted with methanol/acetone 1 1 (v/v).

Isotope determination Aliquots of the chromatographic fractions were counted in a Packard-Tri-Carb liquid scintillation spectrometer After TLC, the radioactive spots were located by autoradiography For quantitation, the optical density of the film was measured as described earlier (9)

RESULTS

I Separation of Labelled Urinary Metabolites

Urinary labelled compounds were adsorbed to Amberlite XAD-2 and quantitatively almost all were eluted with methanol. Aliquots of the residue in the methanol extracts were analysed by TLC and by chromatography on Sephadex LH 20

Thin-layer chromatography

Chenodeoxycholic acid 24 ^{14}C Only small amounts of the labelled compounds appeared at the positions of taurochenodeoxycholic and glycochenodeoxycholic acids (Fig. 1 upper

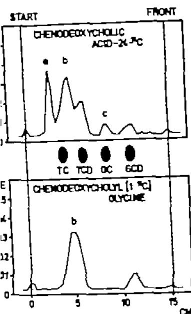


Fig. 1. TLC-separation of urinary labelled metabolites after administration of chenodeoxycholic acid-24-^{14}C (patient AM) and chenodeoxycholyl[1 ^{14}C]glycine (patient JS). Solvent system II. Reference substances: taurocholic acid (TC), glycocholic acid (GC), taurochenodeoxycholic acid (TCD) and glycochenodeoxycholic acid (GCD). *Crosshatching represents spots detected by spraying with phosphomolybdic acid.* Densitometric recording of the autoradiograph is given.

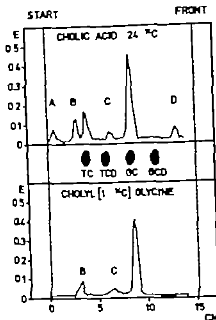


Fig. 2. TLC-separation of urinary labelled metabolites after administration of cholic acid 24-^{14}C (patient HL) and cholyl[1 ^{14}C]glycine (patient HL). For explanation, cf. fig. 1.

part). Most of the isotope was found in two broad peaks, *a* and *b* and smaller amounts in peak *c*.

Chenodeoxycholyl[1 ^{14}C]glycine. Only small amounts of the labelled compound showed the TLC mobility of unchanged glycochenodeoxycholic acid. Most of the isotope consisted of a compound with the mobility of compound *b* (Fig. 1 lower part).

Cholic acid-24-^{14}C. A representative chromatogram is shown in Fig. 2 (upper part). In all 4 patients investigated, most of the labelled compounds were found at the positions of taurocholic and glycocholic acids. Apart from these positions labelled compounds were consistently found in four other regions, A–D.

Cholyl[1 ^{14}C]glycine. Most of the labelled compound appeared as glycocholic acid but labelled compounds also appeared at the positions B and C (Fig. 2, lower part).

Chromatography on columns of Sephadex LH 20

Chenodeoxycholic acid-24-^{14}C and chenodeoxycholyl[1 ^{14}C]glycine. Most of the labelled compounds found after the administration of chenodeoxycholic acid-24-^{14}C were recovered in the methanol eluate (IV) (Fig. 3). The other

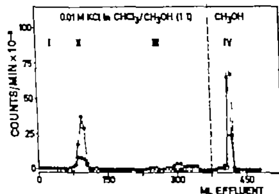


Fig. 3. Column chromatography of urinary labelled metabolites on Sephadex LH 20. Urine extract from patient AM after administration of chenodeoxycholic acid 24-^{14}C (○—○) and from patient JS after administration of chenodeoxycholyl[1 ^{14}C]glycine (●—●).

Table 2. *Distribution of conjugates of cholic and chenodeoxycholic acids after chromatography on columns of Sephadex LH 20*

	Percentage of recovered isotopic bile acid								
	Cholic acid-24-^{14}C				Cholyl [1 ^{14}C] glycine		Chenodeoxycholic acid 24-^{14}C		Chenodeoxycholyl [1 ^{14}C] glycine
Patient	J S	H. L.	C. E.	K. J	K. J	H L	J S	A M	J S.
Chromatographic fractions									
I	1	7	1	6	0	0	20	1	0
II	97	82	94	87	88	92	41	28	37
III	0	0	0	0	0	0	0	1	6
IV	2	11	5	6	12	8	39	70	57

labelled compounds were eluted at the position of tauro- and glycochenodeoxycholic acids (II) Smaller amounts were eluted before (I) and after (III) these compounds. The percentage distribution of isotope between fractions I–IV are listed in Table 2. The major labelled metabolites with TLC mobilities in regions *a* and *b* were eluted in fraction IV Compound *c* appeared in fraction II Chromatography of labelled metabolites of chenodeoxycholyl[1 ^{14}C] glycine separated the unchanged labelled chenodeoxycholyl[1 ^{14}C]glycine in fraction II from the major labelled metabolites which were eluated in fraction IV (Fig 3 Table 2)

Cholic acid 24-^{14}C and cholyl[1 ^{14}C]glycine The methanol extracts after chromatography on Amberlite XAD-2 were rechromatographed on Sephadex LH 20 Most of the labelled conjugates of cholic acid-24-^{14}C were found in a band after the front (II) Smaller amounts appeared with the front (I) and in the methanol eluate (IV) The percentage distribution of isotope in the fractions I–IV are listed in Table 2. TLC of labelled compounds showed that peak II contained labelled compounds with the mobilities of tauro- and glycocholic acids and compounds with the same mobilities as compounds B and C. A labelled compound with the same mobility as compound A was eluted with methanol (IV) The labelled compounds B and C, which were also seen after the administration of cholyl[1 ^{14}C]glycine could not be separated from labelled glycocholic acid by chromatography on columns of Sephadex LH 20

II *Nature of Labelled Urinary Conjugates*

The labelled urinary metabolites were isolated by preparative TLC and again subjected to TLC in different phase systems before and after solvolysis or enzymatic hydrolysis.

Chenodeoxycholic acid 24 ^{14}C and chenodeoxycholyl[1 ^{14}C]glycine

Compound a. Enzymatic hydrolysis and solvolysis yielded labelled compounds with the TLC mobilities of chenodeoxycholate monosulphate and taurochenodeoxycholic acid respectively This compound is probably taurochenodeoxycholate monosulphate.

Compound b Labelled compounds with the same TLC mobility as compound *b* were obtained after the administration of either chenodeoxycholic acid-24-^{14}C or chenodeoxycholyl[1 ^{14}C]glycine. The TLC-behaviour of compound *b* corresponded to that of the glycochenodeoxycholate monosulphate Enzymatic hydrolysis and solvolysis yielded chenodeoxycholate monosulphate and glycochenodeoxycholic acid respectively

Compound c The behaviour of this compound did not correspond to that of a sulphate at chromatography on Sephadex LH 20 However solvolysis yielded a compound with the TLC mobility of glycochenodeoxycholic acid. The nature of this compound was not determined.

Cholic acid 24-^{14}C and cholyl[1 ^{14}C]glycine

Compound A This compound behaved like a bile acid sulphate [illegible]

dex LH-20 After solvolysis its TLC mobility was the same as that of taurocholic acid. This suggested that compound A was a sulphate ester of taurocholic acid.

Compound B This compound was found to be a labelled derivative of glycocholic acid which did not behave like a sulphate at chromatography on Sephadex LH-20. However solvolysis yielded a compound with the TLC-mobility of glycocholic acid. Its nature was not determined.

Compound C Solvolysis of this compound yielded a compound with the TLC mobility of glycocholic acid. At chromatography on Sephadex LH-20 it did not behave like a sulphate ester The nature of the compound was not determined.

Compound D This compound was separated by reversed phase partition chromatography into several compounds. Their natures were not determined.

DISCUSSION

In a study of the metabolism of lithocholic acid-24-^{14}C in man several new labelled solvolysable conjugates were found in bile (9). Palmer (15) eventually identified them as 3- sulphates of lithocholic acid, glycolithocholate and taurolithocholate a new pathway of bile acid metabolism in man being thereby recognized. Previous studies have shown that lithocholic acid-24-^{14}C in infants with extrahepatic biliary atresia is almost entirely excreted in the form of 3-sulphates of tauro- and glycolithocholic acids (12). In the present investigation 40–70 % of chenodeoxycholic acid-24-^{14}C was found to be excreted in the form of sulphates in the urine. After the administration of the labelled glycine conjugate of this bile acid, 60 % of the isotope was recovered in the urine in sulphated form. This indicated the same excretion pattern as that of pure chenodeoxycholic acid-24-^{14}C. In contrast to chenodeoxycholic acid only a minor amount of cholic acid-24-^{14}C was recovered in the sulphate fraction after Sephadex LH-20 chromatograhpy The same excretion pattern was observed after the administration of labelled glycocholic acid.

Both lithocholic and chenodeoxycholic acids have been shown to induce cholestasis in rodents (5 7) This also applies to the glycine and taurine conjugates of lithocholic acid, but the corresponding sulphates were found to have a markedly weaker cholestatic effect (5) Cholic acid has not been shown to be hepatotoxic. Sulphation enhances the urinary excretion of the conjugates of lithocholic acid (16) Further studies are required to clarify whether the renal clearance of the sulphates of other bile acids is higher than that of the corresponding taurine and glycine conjugates.

Unpublished observations suggested that the same degree of sulphation of the urinary metabolites of chenodeoxycholic acid-24-^{14}C occurs both in infants with intrahepatic cholestasis and infants with extrahepatic biliary atresia (13). However sulphation of bile acids appears to occur not merely in liver disease, since sulphate esters of bile acids were isolated in the urine from normal infants during the first week of life as well as in meconium (13) Larger amounts of cholesterol and steroid hormones are excreted sulphated in normal newborn infants as compared to adults (2, 4 6) The high level of sulphate esters of bile acids in the urine of infants with cholestasis may be related to infancy rather than to hepatic diseases.

SUMMARY

The urinary excretion of bile acid conjugates was studied after the administration of 24-^{14}C labelled chenodeoxycholic acid and cholic acid and their corresponding glycine conjugates labelled with glycine 1 ^{14}C in 5 infants with extrahepatic biliary atresia. Chenodeoxycholic acid 24-^{14}C and chenodeoxycholyl[1-^{14}C]glycine were mainly excreted in the form of labelled metabolites with the TLC behaviour of monosulphates of tauro- and glycochenodeoxycholate and glycochenodeoxycholate, respectively Most of the cholic acid-24-^{14}C and the

cholyl[1 ^{14}C]glycine were found to be excreted in the form of glycocholate. All labelled bile acids were excreted in conjugated form

ACKNOWLEDGEMENTS

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AN OUTBREAK OF COXSACKIE VIRUS TYPE B2 AMONG NEONATES IN AN OBSTETRICAL WARD

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The clinical and epidemiological characteristics of Coxsackie B virus infections in newborns, especially in nursery wards, have been described and discussed in several papers during the last 20 years (1 3 (review), 4 5 7 8). Coxsackie B virus infections in early infancy may cause severe and sometimes fatal meningoencephalomyocarditis. Outbreaks of such infections are not frequent but present a number of problems to the clinicians. It therefore appears motivated to describe the experiences encountered in the management of an outbreak of neonatal Coxsackie B2 virus infections in an obstetrical and nursery ward in July 1972.

MATERIAL

Patients. An outbreak of infection in 12 newborn infants (7 boys and 5 girls) was studied (Table 1 *a*). They were all delivered in the same hospital (*V*). The first symptoms of disease appeared at an age of 4 to 47 days. Two of the children were born prematurely and the others were born at term. Fifteen of the infants' parents, demonstrating symptoms of infection, and 89 staff members of the obstetrical clinic were studied epidemiologically and serologically.

Thirty-seven out-patients treated at hospital *V* and 51 staff members of a department of obstetrics and a nursery ward of another hospital (*U*) located in an adjacent city but presumably outside the epidemic area served as control groups for the complement fixation and neutralization tests respectively.

METHODS

Isolation of virus. Throat swabs, fecal specimens and cerebrospinal fluid samples (CSF) were used for virus isolations. From one case autopsy specimens obtained from myocardium, brain, lungs, liver and intestines were tested. Samples of homogenized specimens were inoculated into two cultures of green monkey kidney (GMK), Hep-2 and human lung fibroblast cells, respectively. The cultures were observed for 12 days. Fluid and cells from cultures showing signs of cytopathic changes were passaged once and the agent isolated was then typed. Samples consisting of CSF autopsy material and, in some cases, of fecal specimens were in addition, inoculated intracerebrally and subcutaneously in 1 2-day-old mice. These were examined for 2 weeks. The isolated virus was identified according to conventional techniques.

Serology. Acute phase as well as convalescent phase serum samples, the latter taken 2 to 4 weeks post infection, were analysed by complement fixation (CF) and neutralization (NT) tests. Sera from staff members were tested by a NT-screening procedure using the 1/20 dilution only.

RESULTS

Clinical observations

A rise of temperature to 38–39 C and failure to thrive were the first symptoms suggesting infection. The temperature remained slightly elevated up to 4 days. During this time several of the children were sleepy and had difficulty nursing. After this period their general condition improved rapidly. In 9 of the children the disease had a mild course. After 2 or 3 days of moderate fever and 5 to 6 days when reduced motor activity and feeding problems were recorded the children improved markedly and they were sent home after 2 weeks in hospital. Clinical and labora

Table 1a *Clinical and laboratory findings*

Case no	Date of birth	Sex	Birth weight/ gestation (grams/weeks)	Day of onset	CSF values Leuko-cytes per mm³	Poly %/ mono	Protein (mg %)	Glucose mg % CSF/blood
1	720713	F	3 300/38	7	—	—	—	—
2	720714	F	2 600/38	6	628	73/27	100	50/80
3	720714	F	3 000/38	6	576	72/28	136	60/90
4	720716	M	3 260/41	4	84	33/67	190	40/80
5	720717	M	3 180/39	7	923	28/72	150	40/50
6	720714	F	3 190/38	8	688	73/27	125	30/40
7	720715	M	3 100/39	7	222	31/69	300	30/80
8	720714	F	4 820/42	10	269	44/56	70	60/80
9	720714	F	2 650/37	6	184	3/97	370	40/90
10	720713	M	2 660/40	14	222	44/56	170	40/60
11	720610	M	1 400/29	47	345	76/24	156	30/70
12	720717	F	1 700/31	14	87	24/76	90	20/40

tory findings are summarized in Table 1 *a* and 1 *b*.

The mother of case 1 showed signs of disease as fever, headache and nausea at admission to the delivery ward. These symptoms disappeared soon but she was again hospitalized a week later since signs of endometritis were observed. The child was therefore cared for at a nursery from the age of 7 days. A general floppiness was observed at the time when the infant was discharged from the obstetrical ward and in the nursery the infant did not thrive properly. At the age of 13 days the child died. The histopathology showed disseminated acute myocarditis and leptomeningitis. Several organs demonstrated signs of impaired blood circulation. The myocarditis was considered responsible for the fatal outcome.

Case 11, an infant boy nursed at the premature ward of the pediatric clinic showed failure to thrive. Symptoms of infection became obvious on the 48th day. A moderate pleocytosis was observed in CSF. Five days later a sudden deterioration occurred with circulatory insufficiency. X-ray examination showed increased heart size and the ECG indicated a myocarditis. The infant was digitalized and treated in a respirator for 5 days. Convulsions occurred during this period but after 11–12 days of treatment the child improved.

The first 5 diseased infants were treated with high doses of ampicillin and gentamicin on the basis of suspect coli infection until after 5 days the viral etiology was known.

A follow-up examination four months after discharge from the hospital revealed normal neurological development in 9 of the children while 2, cases 11 and 12, lagged behind, as expected, due to prematurity.

Fifteen of the 24 parents reported symptoms of disease mainly fever, headache and myalgia. One of the staff of the nursery ward of hospital *V* fell ill with fever and intense headache. She was admitted to a clinic for infectious diseases. CSF was normal but ECG showed signs of perimyocarditis.

Virology

Coxsackie virus type B2 was isolated from 8 children—in three cases from CSF—and from 2 parents. In 5 cases virus was isolated from more than one type of specimen. From 2 cases virus was isolated in mice but not in cell cultures.

From the dead child (*case 1*) Coxsackie virus type B2 was isolated from the meninges, cerebral parenchyma, myocardium and lung tis-

able 1b *Clinical findings*

▪▪▪).	History	Course
1	Low muscle tone when discharged from hospital after birth. Spoon feeding. Sleepy and quiet	Mors subita infantum
2	First born of twins. Low grade recurrent fever 5 days. Irritability Not gaining weight	Normal temperature after 5 days. Somewhat difficult to feed. Uneventful recovery
3	Second born of twins. Low grade recurrent fever 5 days. Pneumonia suspected on X-ray	Normal temperature after 5 days. More difficult to feed than her sister Uneventful recovery
4	Sudden fever on 7th day Increasing anorexia. Sleepy	Normal temperature after 3 days. Feeding difficulties 4 days. Uneventful recovery
5	Mother had mild toxicosis. A second fall in weight. Diarrhea. Sluggish. Urinary tract infection	No fever from 4th day Scaling skin. Feeding problems
6	Poor gain in weight Vomiting after meals No fever	Improvement after first day No fever
7	Almost impossible to feed full meals. Apathy Whimpering cry	Very difficult to feed. Subnormal temperature for 2 days. After 3 days rapid improvement
8	Suddenly turned blue on 13th day Later on sluggish, feeding slowly	Irritability increased muscle tone and fever first 2 days at hospital. Uneventful recovery
9	Feeding problems at home. Apathy Mother treated for hydronephrosis during pregnancy	No feeding difficulties from 2nd day Uneventful recovery
10	Feeding problems and apathy at home. Poor gain in weight Referred from well baby clinic to hospital	First 3 days difficult to feed. Sleepy Fever one day Uneventful recovery
11	Premature birth at 29 weeks. Unexpectedly sleepy on 47th day not accepting formula. Shared room with case 5	Sudden heart incompensation on 7th day General convulsions 2 days later Respirator 5 days. Thereafter slow improvement
12	Premature birth at 31 weeks Asphyxia. Intubated. IRDS. Frequent attacks of apnea. Artificial ventil Shared room with case 5	Low muscle tone. Artificial respiration for 2 and 5 days. Slow and steady improvement.

me. No virus was isolated from specimens of staff members.

Serology

Nine of the patients and 2 of the parents demonstrated a significant rise in CF antibodies against a Coxsackie B2 antigen. The fatal case had no CF-antibodies against the antigen detectable in the serum specimen taken at the autopsy Five of the children demonstrated significant rise of neutralizing Coxsackie B2 antibodies. In 2 of the children no antibodies were demonstrable, one of these was the fatal case 1 the other was one of the two prematurely born children (case 12)

Although no virus was isolated from staff members, results of CF and NT revealed that Coxsackie B2 infections apparently had occurred among the personnel (Table 2). An inquiry to estimate the frequency of symptoms like fever and headache with or without pharyngitis or enteritis during and after the outbreak of infection among the neonates supported this assumption. The results of the

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A follow up examination four months after discharge from the hospital revealed normal neurological development in 9 of the children while 2, cases 11 and 12, lagged behind, as expected due to prematurity

Fifteen of the 24 parents reported symptoms of disease mainly fever headache and myalgia. One of the staff of the nursery ward of hospital V fell ill with fever and intense headache She was admitted to a clinic for infectious diseases. CSF was normal but ECG showed signs of perimyocarditis.

Virology

Coxsackie virus type B2 was isolated from 8 children—in three cases from CSF—and from 2 parents. In 5 cases virus was isolated from more than one type of specimen. From 2 cases virus was isolated in mice but not in cell cultures.

From the dead child (*case 1*) Coxsackie virus type B2 was isolated from the meninges, cerebral parenchyma, myocardium and lung tis-

mon. As a rule the virological diagnosis can be obtained within 3–4 days.

Moreover during the epidemic season, Coxsackie B infections should always be thought of in connection with infants demonstrating signs of infection and, in particular in children with meningoencephalitides or sudden heart incompensations. The acute phase of myocarditis in the newborn may require digitalization and artificial respiration. When the acute phase has been overcome recovery may follow after some few days. Impaired heart function as a late sequelae has not been reported (2), and meningoencephalitis caused by Coxsackie virus does not seem to have any adverse effects on the neurological development (9).

SUMMARY

An outbreak of Coxsackie B2 virus infections in 12 neonates was studied. The source of the infection seemed to be the mother of the first diseased baby who later spread the infection in the ward, and although no virus carrier was found among the staff members there was serological evidence that nearly 40% of the personnel had been infected during and the month following the infection of the babies. The need for careful observation of patients and personnel presenting signs of infection especially when Coxsackie virus infections are common is stressed.

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	CF-antibody		Neutralizing antibody	
	Significant rise	Titre >32	Titre >20	Titre <20
Medical staff	9 (13 %)	8 (11 %)	23 (33 %)	47 (67 %)
Controls	3 (8)	1 (3 %)	2 (4 %)	49 (96 %)

inquiry demonstrated that 22 of the ward personnel had reported illness during the period of the outbreak and the following 4 weeks. Thus, there was serological as well as anamnestic evidence for Coxsackie virus infections of the medical staff

DISCUSSION

Outbreaks of Coxsackie B infections in newborn nurseries have been described from several parts of the world The present report describes an outbreak of infection caused by Coxsackie virus type B2 Twelve newborn babies fell ill soon after they left the obstetrical ward, all with signs of aseptic meningitis and in two cases also of myocarditis. One child died and the post mortem examination revealed a meningoencephalomyocarditis.

Difficulty in rapid tracing of the source of a nursery outbreak so that e g virus carriers can be isolated are well illustrated by the present report The source of infection could not be conclusively stated although a fairly extensive epidemiological study was done. None of the staff members was excreting virus at the time when the samples were collected, i e the first 2 weeks after the onset of the outbreak.

However in an inquiry 22 out of 89 of the staff members later reported symptoms of illness not incompatible with a Coxsackie virus infection during the time for the outbreak and the following month and the serological study (Table 3) suggested that a Coxsackie B2 virus infection might have occurred in 40% of the staff Nevertheless the distribution of cases within the obstetrical ward as well as other epidemiological and anamnestic information indicated that the source of infection probably was to be found among the patients and not among the personnel. The earliest history of infection appeared to be that of the mother of the child which died She seems to have spread the infection at least to her baby and to another mother with whom she was sharing a room. The baby seems to have spread the virus, perhaps via personnel, to the other infants.

As is the case for most enterovirus diseases subclinical infections are common in local outbreaks of Coxsackie B virus infections (4 6) and the symptoms of the clinically overt infections may be mild and diffuse (10) The physician in charge of the nursery is therefore often not aware of a prevailing Coxsackie virus epidemic The first cases among the newborns may—as occurred in the present outbreak—clinically suggest a bacterial meningitis and thus delay laboratory diagnosis and adequate isolation precautions. Increased numbers of virological examinations of patients and personnel of delivery wards and nurseries in the late summer/fall period would result in early detection of virus carriers and reduce the risk of spread of infection to neonates. Routinely performed virological screening tests on personnel attending nursery wards and on the pregnant women when admitted to the delivery wards may thus, be wise in periods when enterovirus infections are com

Table 3 *Medical staff members distributed according to probable infection and serological evidence of Coxsackie infection*

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Yes	20	9
No	51	20

* In 18 of 89 staff members no second serum specimen was available and these were therefore excluded. Only 2 belonged to the group reporting illness.

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Table 1 *Means and ranges of family income and expenses in Lebanese pounds*[a]

	Thriving	Failing to Thrive
No. of families	40	30
Monthly income	380 (190–900)	274 (120–450)
Total monthly expenses	384 (215–732)	315 (131–463)
Food	188 (103–325)	164 (64–340)
Housing	86 (13–272)	73 (8–204)
Other	110 (28–215)	78 (12 149)

1 Lebanese pound = approximately 30 U.S. cents at time of study

the Thriving families is three times as great as that of the Failing to Thrive families. None of the Failing to Thrive families owned a car whereas 7 Thriving families owned cars.

(i) *Houses*

Thirty percent of the Thriving families owned their residences. The values of these homes ranged from 500 L.L. for a hut built upon common land to 49 000 L.L. for two modern flats built of concrete bricks and rendered with cement, well painted, comprising five rooms, kitchen, bathroom and two balconies in each. Twenty percent of the *Failing to* Thrive families owned their homes. Electricity and water supply were available in almost all houses, except two of the Failing to Thrive families who used kerosene and had to share outside water supply with one or more other families. Considering the mean number of children in the two groups, the Failing to Thrive

Table 2. *Percentage of total expenditure on different food items*

	Thriving	Failing to Thrive
Mean for the Group (L.L.)	188	164
	%	%
Dairy products	17 0	15.2
Eggs, meat and poultry	25 0	18.9
Vegetables and fruits	18.1	18.9
Cereals and legumes	18.6	22.6
Others	21.3	24.4

Table 3 *Mean and range of values of family possessions in Lebanese pounds in Thriving and Failing to Thrive families*

	Thriving	Failing to Thrive
No. of families	40	30
House and Other residence	4 874 (0–50 000)	600 (0–12 000)
Households goods	3 972 (418–20 585)	1 608 (143–6 279)
Motor car	908 (0–12 000)	0
Total	9 754 (418–70 585)	2 208 (143–13 763)

homes were more crowded and more poorly kept.

(ii) *Household goods* (Table 4)

(a) *Furniture* that is including beds, mattresses, blankets, floor carpets, chairs, tables, and other objects that furnish the bedroom, dining room and sittingroom and excluding those mentioned in the next paragraphs or kitchen equipment. Nineteen Thriving families (47%) had a complete livingroom-set, fourteen of them (35%) had complete bedroom-set and 3 families (7%) had complete diningroom-set. *Whereas only 3 Failing to Thrive families* (10%) owned complete livingroom-set, 2 had *complete bedroom-set* (6%) and none had complete diningroom-set.

There were 94 beds (2.4 per family) plus 67 mattresses (approximately 2 per family) available in the Thriving homes in comparison with 42 beds (1 4 per family) plus 63 mattresses (2.1 per family) in the Failing to Thrive. In most of the households father and mother sleep in bed with the younger children. Older children sleep on mattresses on the floor In small apartments beds were used during the day for sitting, for sorting laundry and other purposes.

Furniture values comprised 42.2% of the household objects and 15 8% of the total family possessions in the Thriving group whereas it composed 49 0% and 25.5% respectively in the Failing to Thrive group.

Acta Pædiat Scand 63 108–112, 1974

FAILURE TO THRIVE IN LEBANON

III *Family Income Expenditure and Possessions*

ABDALLAH A KANAWATI OLFAT DARWISH and DONALD S. McLAREN

From the Nutrition Research Laboratory School of Medicine American University of Beirut Beirut Lebanon

Malnutrition is associated with low income and as family income increases, total expenditure on all kinds of food increases (6 8) However the percentage of total expenditure for certain foods, especially cereals and starches, decreases as income increases Family income tells us little about how people actually live and in the hope of gaining new insights into the nature of poverty a study was also made of the possessions of low socio-economic families similar to that carried out by Lewis (5) in Mexico City

MATERIAL AND METHODS

Seventy Lebanese Arab Moslem families were investigated Forty had a young child who was "Thriving and thirty had a child who was "Failing to Thrive" as defined previously (2, 3). These contrasting groups of families were all of low socio-economic class and were located as follows. *Basta* (down-town Beirut) 13 Thriving 12 Failing to Thrive *Bourj al Barajne* (suburb of Beirut) 27 Thriving 18 Failing to Thrive. The average number of children was 6 in the Thriving and 7 in the Failing to Thrive group.

The parents of each family were questioned by one of us (O D) concerning all sources of income and approximate amount the amount of expenditure on housing, furniture, home appliances, luxury items, clothes, food (in various categories) and miscellaneous expenditure.

An inventory of the possessions of each family was taken and certain other information obtained such as age of possessions, original purchase price, approximate present replacement value, how items were obtained (bought new or second-hand or gifted) how people financed their purchases, where they did their shopping and the condition of the possessions.

RESULTS

Income and Expenses

Monthly income in the two contrasting groups is markedly different (Table 1). Thriving families were almost in a state of financial balance whereas Failing to Thrive families had an average monthly deficit of 41 L.L.

Table 1 shows also the distribution of family monthly expenditures. Both groups of families spent equal proportions of their income on housing i.e rent property taxes, electricity and water food and other items such as transportation children s education clothes, cleaning and medications.

In Table 2 monthly expenditure on various food items is shown. The proportion that was spent on superior foods such as dairy products, eggs or meat and fish tended to be higher in the Thriving group than the Failing to Thrive. On the other hand less expensive foods such as cereals and legumes and certain other foods were more consumed by the poorer Failing to Thrive families.

Total Possessions

The mean values and ranges of total family possessions including values of owned houses or other residences, household goods and motor cars (whether they were for public use or for family private use) are shown in Table 3 It is clear that the value of the possessions of

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the poorer Failing to Thrive group and rather more on cheaper cereals and legumes and other items. An approximate calculation was made of the average daily intake of protein and energy per head from data on family monthly food expenditure, taking into consideration the prevailing prices and the local meal pattern. The recommended allowances per head per day were calculated according to the age and sex distribution in Lebanon (9) and also the National Research Council Recommended Daily Allowances (7). In this way it was estimated that the Thriving group could receive for their expenditure on food an average of 1 748 kcal and 40.1 g protein per head per day. The comparable figures for the Failing to Thrive group were 1 537 kcal and 36.8 g protein. The recommended daily allowances per head are 2 120 kcal and 53.6 g protein. Thus both groups appear to be deficient in both energy and protein intake, the Failing to Thrive rather more so than the Thriving. Using the National Research Council standards the deficit is greater for protein than energy but these probably overestimate the protein needs of our population.

The total value of possessions (Table 3) contrasts greatly in the two groups with the Thriving having possessions that are, on the average, more than three times as valuable as those owned by the Failing to Thrive group. This suggests that the former families are more "successful" in many ways and do not suffer at least in terms of child health, from spending more on possessions. The contrast between the two groups is least for household goods, more for houses, and most for motorcars, which are owned solely by the Thriving group.

More detailed consideration of household goods (Table 4) shows that the value of those of the Thriving group is about twice that of the Failing to Thrive group for every category except the last (decorations, jewelry and toys) where the figure is nearly ten times greater. The data for toys are especially revealing. Six Thriving families and only one Failing to Thrive had any toys and the average amounts of money spent on these were only 118 L.L. and 35 L.L. respectively. It is clear that the home environment of the preschool child in these communities is very impoverished with regard to stimulation.

Table 5 shows contrasts, between the two groups most marked in the case of refrigerators, radios, heaters, complete living rooms, etc.

We were able to collect information of a general nature that gave some insights into the way in which these people attempt to overcome their problems. Failing to Thrive families (47.7%) changed their addresses during the previous two years more frequently than the Thriving group (22.6%) and $0.05 > p > 0.02$. This mobility is probably a consequence of their insolvency. Most families bought their food and clothes on a charge account basis from neighbourhood shops. They frequently borrowed money from relatives and neighbours. Emigration was not infrequent. The father is the main support of the family but some mothers who were widows or divorced filled this role and occasionally young children earned a little money to help their families. The lack of funds often drove people to make their own goods such as clothes as illustrated by the possession of sewing machines, or furniture.

SUMMARY

Information was obtained from 40 Thriving and 30 Failing to Thrive families, as previously defined, living in two low socio-economic areas of Beirut concerning their income, expenditure on various items and their possessions. Both groups were unable to save money but the Failing to Thrive were usually in debt. This group spent proportionally more on food and bought less nutritious food. Families of neither group were fully able to meet their energy or protein requirements.

The possessions of the Thriving group were worth, on average, about three times those of the Failing to Thrive group. The contrast

(b) *Home comfort appliances.* These included refrigerator heater sewing machine washing machine and electric iron. The mean values of these items was much higher in the Thriving group than the Failing to Thrive. Home comfort appliances composed 27 9% of household goods and 10 3% of total family properties in the Thriving group (Tables 3 and 4) Similarly in the Failing to Thrive group comfort appliances composed 27 7% of the household goods and 14 5% of their total properties. Percentages of families who owned such appliances are shown in Table 5 Differences between the two groups were analysed using the χ^2 test Refrigerators, radios and heaters were much commoner in the Thriving families ($p<0\ 001$) as were televisions and washing machines ($0\ 05>p>0\ 02$) Both groups owned similar numbers of sewing machines and only one Thriving family owned a knitting machine Sixteen Thriving families had some decorative objects such as chandeliers pictures, vases artificial flowers, wall carpets and curtains whereas only 5 Failing to Thrive families had decorative objects. Eight families of the better off group had provided their children with some toys to play with i.e bicycles, in contrast with the other group where only one family had such toys

Expensive items such as bedroom set or livingroom set refrigerator and television were mostly purchased by monthly instalment. Kitchen equipment such as utensils, gas stoves were usually received as gifts and two families of the Thriving group received a refrigerator from close relatives. Furniture was in most cases bought at marriage and kept in fairly good condition depending on the duration of marriage Few of either group had recently (2 months – 2 years) renewed some of their furniture.

Table 4 *Mean and range of values of various household goods in Lebanese pounds in Thriving and Failing to Thrive groups*

Category	Thriving	Failing to Thrive
No. of families	40	30
Furniture	1 762 (305–10 355)	789 (60–4 000)
Home comfort appliances	1 078 (0–3 290)	489 (0–1 804)
Radio–T V., Recorder	561 (0–2 825)	169 (0–900)
Kitchen equipment	295 (30–1 000)	133 (19–540)
Decorations, jewelry toys	276 (0–4 725)	29 (0–285)
Total	3 972 (418–20 585)	1 608 (143–6 279)

Table 5 *Percentage of families in Thriving and Failing to Thrive families owning certain possessions*

	Thriving	Failing to Thrive	p
No of families	40	30	
Refrigerator	95.0	56.7	<0.001
Radio	92.5	60.0	<0 001
Heater	85.0	46.7	<0.001
Sewing machine	60.0	43.3	N.S.
Television	50 0	20.0	$0.02>p>0.01$
Complete living room set	47.5	10.0	<0.001
Complete bedroom set	35 0	6.0	$0\ 001<p<0.01$
House or other residence	30 0	20.0	N.S.
Jewelry	27.5	10 0	N.S.
Washing machine	20 0	3.3	$0\ 05>p>0.02$

DISCUSSION

In a previous study carried out about 3 years before the present one on a larger sample of families, we reported a higher family income among the Thriving than the Failing to Thrive group (4) This was also true in the present study

The figures for expenditure (Table 1) suggest that while the two groups spend approximately similar proportions on the main categories (food, housing and other) the Thriving group is just "making ends meet" with no savings, while the Failing to Thrive families tend to be in considerable debt.

Figures for expenditure on food (Table 2) show a smaller proportion spent on expensive but more nutritious animal-derived food by

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DIAGNOSTIC VALUE OF MANNITOL-INDUCED DIURESIS IN CHILDREN

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Hypertonic saline infusions or hydropenia, the classical procedures used to assess renal concentrating ability (6, 18, 27, 7, 9, 20) are often hazardous to apply in the pediatric age group, especially if the patients are very young. The maintenance of a dry diet as well as a high saline load are frequently badly tolerated in infancy, own to the extreme hydrolability of this age group and to the inability to excrete efficiently a large sodium load. Baldwin (2) and later on other authors (1, 5, 33) have introduced the use of hypertonic mannitol infusions instead of a saline load. This substance is filtered in the glomerulum but is not re-absorbed in the nephron. The load of osmotic active substance is therefore more rapidly excreted. The same authors still insist however in a period of fluid restriction during the 10 hours before the test.

A modified technique of mannitol-induced diuresis without a preparative hydropenic period has been in use for 3 years in the University Children's Hospital of Berne to study all patients who needed examination of their renal concentrating ability. Its diagnostic value is analysed. The study procedure was perfectly well tolerated, and no complications have been seen. It is possible to use this test in a systematic way in hospitalized patients as well as on an ambulatory basis.

METHODS

26 children were examined for renal concentrating ability. 7 were judged to be "normal" since in these patients no polyuria or polydipsia existed and glomerular filtration rate (GFR) was normal. One patient with polyuria who turned out to be potomaniac was also included in this "normal group". All other patients with polyuria and polydipsia were classified as pathologic cases. They showed a variety of clinical diagnosis known to produce polyuria and polydipsia. For precise diagnoses, see Results (Tables 2 and 3).

Prior to the study the patients were neither fasted nor was fluid intake restricted. Thus the individual fluid balance was not changed by any preparative measures. The patients received a light breakfast and were kept on bedrest in the metabolic ward during the test period, which lasted about 2 hours. Two indwelling needles or i.v. catheters were placed into peripheral veins, one for infusion of the test substances, one for repeated blood-sampling. This catheter was flushed after each sampling with slightly heparinized saline solution, and then it was tightly clamped. A bladder catheter was placed with its tip at the meatus internus of the urethra and was flushed with air at each closing of a urine period. No fluid was used to flush the catheter.

After collection of blood and urine for blank values of inulin and PAH, a priming dose of 1 ml inulin (10% /kg body weight) and of 0.06 ml PAH (20%/kg body weight) was given intravenously. Thereafter inulin and PAH were infused in amounts

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between the groups was least for household goods greater for houses, and greatest for motorcars. The Failing to Thrive group had far fewer decorations, jewelry, and toys and the latter may be an important index of the richness of the child s environment. Refriger ators, radios and heaters were especially prized

ACKNOWLEDGEMENTS

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...ality (.../kg H_2O) Plasma	C_{osm} (ml/min/1 73 m²)	$T^c_{H_2O}$ (ml/min/1 73 m²)	Urine volume (ml/100 ml GFR)	C_{osm} (ml/100 ml GFR)	$T^c_{H_2O}$ (ml/100 ml GFR)
285.2	4.54	2.81	1.32	3 46	2.14
288.7	9.98	5 04	3.36	6.79	3 43
290.5	9.12	4 18	3 83	7 07	3.24
291.5	7 49	3.29	5	8.91	3.91
292.4	15 7	6.64	5.77	10	4 23
...Pitressin 8 mU/kg b.w./h					
293 4	15 3	6.24	6.97	11 77	4 80
294.3	9.5	3 57	8.59	13 76	5 17
294 7	16.8	6 18	8.92	14 11	5 19
295 2	19 7	6.86	9.80	15 03	5.23

...evenly increasing urinary volumes as well as osmolar clearances and values for free water re-absorption during the study

In Fig. 1 are plotted the values obtained ...m 9 *osmolar clearances performed in the 7* "normal" children seen in Table 2. Each 7–9 points correspond to one study. The values before (open signs) and after administration of vasopressin (closed signs) are clustered to give a uniform picture of increasing TH_2O with increasing urinary volume. The cross-hatched area of this figure comprises the mean ±1 S.D for all values, the hatched area includes the mean ±2 S.D

In Fig. 2 are tabulated all values of positive *but subnormal TH_2O. They correspond to the* osmolar clearances of the patients in Table 2. Although these patients are all producing a concentrated urine they present some clinical signs of a disturbance of the urinary concentrating mechanism. As can be seen, before administration of vasopressin (open signs) the

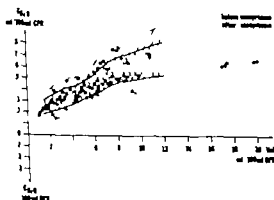


Fig. 1. TH_2O as a function of urinary volume in 7 normal patients submitted to hyperosmolar mannitol infusion. Compilation of all data of 9 studies. Open signs = values obtained before, closed signs = values obtained after exogenous vasopressin. The cross-hatched area comprises mean ±1 S.D, the hatched area mean ±2 S.D

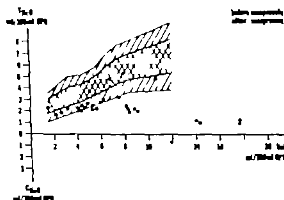


Fig. 2. TH_2O as a function of urinary volume in 7 patients with positive, but subnormal values. Compilation of data of 7 studies. Open signs = values obtained before, closed signs = values obtained after exogenous vasopressin. The hatched area comprises mean normal TH_2O ± 2 S.D

Table 1 *Representative clearance study during mannitol induced diuresis in a normal child (M F, 8 years)*

Clearance period	Time (minutes)	Urine volume (ml/min/1 73 m²)	C_{in} (ml/min/1 73 m²)	C_{PAH} (ml/min/1 73 m²)	FF	Plasma Na (mEq/l)	Plasma K (mEq/l)
	0	Priming injection of inuline and PAH					
		Sustaining infusion I Infusion rate 1 ml/m²/min, with inulin and PAH					
	50	Sustaining infusion II Infusion rate 6 ml/m²/min, with inulin and PAH, Mannitol 10					
1	51–60	1 73	131	589	0.22	139	3.94
2	61–66	4 94	147	693	0.21	138	3.91
3	67–71	4 94	129	589	0.22	137	3.90
4	72–76	4.20	84	417	0.20	137	3.95
5	77–82	9 06	157	741	0.21	137	4 04
	83	Sustaining infusion III Infusion rate 6 ml/m²/min, with inulin and PAH Mannitol 10					
6	83-91	9.06	130	584	0.22	137	4 15
7	92–96	5 93	69	316	0.22	136	4.23
8	97–101	10 6_	119	536	0.22	135	4.28
9	102–106	12.84	131	634	0.21	135	4.32

calculated to maintain constant levels of 50 and 2 mg% respectively throughout the study Equilibrium concentrations were usually achieved after a period of 45 minutes. The infusion rate never exceeded 1 ml/min/m during this period (28). Once equilibrium plasma concentrations for inulin and PAH were obtained the infusion was replaced by a mannitol infusion containing mannitol 10% inulin and PAH as mentioned sodium (75 mEq/l) and potassium calculated to deliver 5 mEq/h/m The infusion rate was set at 6 ml/min/m

During mannitol infusion at least 3 urine samples of a few minutes duration each were collected Blood samples were obtained every 15 minutes. Serum concentrations at the midpoint of each urine sample were read from a concentration/time graph.

After measuring concentrating ability without exogenous antidiuretic hormone vasopressin (Pitressin tannate Parke Davis) was added to the otherwise unchanged infusion in amounts calculated to deliver 8 mU/h/kg body weight. The collection of urine and blood samples was continued with the same rhythm as stated before Blood pressure was frequently controlled

A very careful fluid balance was continuously maintained during the study Intake had to match urinary output, if necessary water supplements were given orally

Osmolar clearances are expressed as every clearance

$$C_{osm} = \frac{U_{osm} \ V}{P_{osm}}$$

Re-absorption or clearance of osmotically free water can be calculated.

If $U_{osm} > P_{osm}$ $C_{osm} > V$ $C_{osm} - V = T_{H_2O}$,
T_{H_2O} is equivalent to re-absorption of free water

If $U_{osm} < P_{osm}$ $C_{osm} < V$ $V - C_{osm} = C_{H_2O}$,
C_{H_2O} is equivalent to formation of free water

If $U_{osm} = P_{osm}$ $C_{osm} = V$

There is neither re-absorption nor formation of osmotically free water

Therefore a negative value of TH_2O is equivalent to a positive value of CH_2O

The data will be presented as plotting of TH_2O (or CH_2O) against urinary volume Each study will furnish between 8 and 10 single values.

The amount of filtrate determines to a considerable degree the absolute rate of free water reabsorption or free water formation (31). In order to compare the values of all patients as well as to correct for uneven results by accidentally false collection of urine the data were corrected for 100 ml of GFR.

Osmolality of urine and plasma was estimated by measuring freezing-point depression of the solution, using an advanced osmometer instrument. Inulin was determined according to the anthron method of Davidson & Sackner (8) and PAH with the method of Bratton & Marshall according to the modification of Richterich (30). Concentrations of sodium and potassium were determined using an Eppendorf flame photometer

RESULTS

Table 1 presents a detailed tabulation of results in a representative study It is stressed that plasma sodium and potassium levels remain constant throughout the study The filtration fraction also remains constant However the clearances of inulin and PAH were out of line in two periods, as were the corresponding urinary volumes. In these periods collection of urine might not have been complete. Correction for 100 ml GFR shows

Table 3 *Clinical diagnosis, form of diuresis filtration rate and free water re-absorption before and after vasopressin in 17 patients with abnormal renal concentrating ability*

Patient	Age (y.)	Age (mo)	Body surface (m^2)	Clinical diagnosis	Polydipsia Polyuria	GFR (ml/min/ 1.73 m^2)	T_{H_2O} (ml/100 ml GFR) before vasopressin	T_{H_2O} (ml/100 ml GFR) after vasopressin
Group 1 Positive subnormal values of T_{H_2O}								
(a)								
C. J	6	3	0.89	Tumor in region of third ventricle	+ −	120	3.03	5.30
M. M.	12		1.18	Central DI, treated with Tegretol[a]	+ −	112	1.34	3.81
W. S	16	10	1.14	Central DI, treated with Tegretol[b]	+ −	116	2.38	6.33
(b)								
M. N.	4	11	0.76	Medullary cystic disease	+	40	1.22	1.42
M. S.	2	2	0.52	Medullary cystic disease	+	—	0.34	0.47
L. L.	12		1.09	Sickle cell disease	+	120	2.70	2.76
R. N.		5	0.29	Bartter's syndrome	+	120	‑.11	2.21
Group 2. Negative values of T_{H_2O}								
(a)								
M. R.	8		0.88	Central DI—post-traumatic	+	113	−7.03	2.33
H. L.	2	6	0.59	Central DI—familiar	+	110	−3.87	5.43
M. M.	11	8	1.18	Central DI—craniopharyngeoma	+	112	−4.30	1.73
W. S.	16	7	1.14	Central DI—craniopharyngeoma	+	116	−3	6.10
A. L.	12	8	1	Central DI—craniopharyngeoma	+	55	−6.20	7
T. W.	12	9	1.04	Central DI—intracranial tumor	+	90	−1.58	3.37
(b)								
S. F.	1	7	0.41	Nephrocalcinosis	+	46	−1.42	−2.07
C. B.	8	8	0.93	Nephrocalcinosis	+	67	−1.48	−1.16
P. L.	12	8	1.40	Chron. glomerulonephritis, hydronephrosis	+	70	−2.15	−1.43
H. S.	7		0.71	Renal DI	+	110	−8	−6.71
M. D.	4	8	0.55	Cystinosis	+	50	−4.86	−5.34
B. H.	10		1.15	Oligomeganephronic hypoplasia	+	60	−1.80	−2.72

[a] Tegretol: 5-Carbamyl-5H-dibenzo [b f] azepin.
[b] The patients are also included in group 2 a, there *before* treatment with Tegretol.
[c] Measurement of GFR was not possible.

(ADH). The vasopressin given exogenously during the second part of the test causes a maximum antidiuretic effect, if endogenous ADH should be insufficiently produced. In all cases the studies were well tolerated.

In the presented studies no attempt was made to determine a maximum value for free water re-absorption. Earlier studies on the formation of a concentrated urine during increasing osmotic diuresis, had suggested a fixed maximum value of TH_2O which was interpreted to demonstrate a saturable capacity of transport in the ascending limb of Henle's loop (39). More recent studies, however, have led to a contrary view. Using hypertonic saline solution to produce the osmotic diuresis, Goldberg et al. (15) have shown that TH_2O continues to rise within attainable limits of solute excretion. A maximum free water reabsorption in mannitol diu-

Table 2 *Tabulation of diagnoses form of diuresis filtration rate and free water reabsorption before and after vasopressin in 7 patients with normal concentrating ability*

Patient	Age (y)	Age (mo)	Body surface (m^2)	Clinical diagnosis	Polydipsia Polyuria	GFR (ml/min/1 73 m^2)	T_{H_2O} (ml/100 ml GFR) before vasopressin	T_{H_2O} (ml/100 ml GFR) after vasopressin
H B	13	7	1.50	Epilepsy	–	112	3 68	5.28
W G	12	10	1.30	Epilepsy	–	105	4 49	6 46
P F	12	7	0.85	Pituitary dwarfism	–	118	6.42	7 73
F V	12	7	0 96	Pituitary dwarfism	–	120	3 87	4 92
P V	2	7	0.28	Pituitary dwarfism	–	94	7.33	7.38
G F	8	8	1	Potomania	+	122	4 11	4.97
M F	8		0 70	Cochayne s syndrome	–	122	4.23	5.23

values of TH_2O are found below 1 S D of the mean normal value If vasopressin was added to the otherwise unchanged infusion some values of TH_2O now reached the band of 1 S D of the mean (closed signs) The single cases cannot be separated in Fig 2, but they are seen individually in Table 3 group 1 a (3 patients) In contrast other values of TH_2O do not increase after administration of vasopressin. These values correspond to the 4 patients presented as group 1 b of Table 3

Fig. 3 is a presentation of all values of those patients who were unable to produce a positive TH_2O when mannitol was infused before addition of vasopressin (patients of group 2, Table 3)

When vasopressin was added two groups can again be differentiated those who now are able to produce a positive TH_2O and those who do not From Table 3 group 2 a it can be seen that after vasopressin 6 patients are able to elaborate a concentrated urine, whereas 6 other patients (group 2 b) remain with hypotonic urine despite the presence of vasopressin.

DISCUSSION

Mannitol infusion induces diuresis without any preparative hydropenic period It increases plasma osmolality and thereby stimulates secretion of endogenous antidiuretic hormone

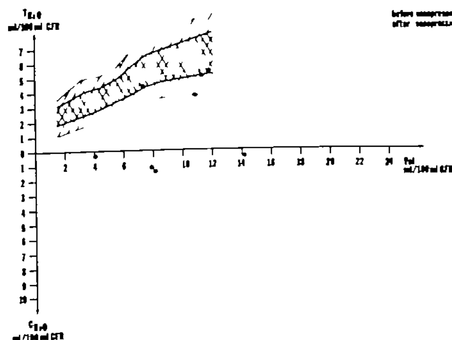


Fig 3 CH_2O and TH_2O as a function of urinary volume in 12 patients with diabetes insipidus. Compilation of all data of 12 studies. Open signs = values obtained before closed signs = values obtained after exogenous vasopressin The hatched area comprises mean normal TH_2O ± 2 S.D

halamo-pituitary system in stimulating secretion of small amounts of ADH in some patients with central diabetes insipidus (13). It is conceivable that some antidiuretic activity was induced in the 2 patients but the amount was insufficient to allow for an adequate response to the stimulus of mannitol diuresis. Exogenously administered vasopressin was necessary for fully adequate response.

Partial renal diabetes insipidus
(Table 3 group 1 b)

The renal handling of free water in the 4 patients was similar to cases with renal diabetes insipidus, though some free water re-absorption was still possible. In contrast to the former group, their values for TH_2O did not increase despite administration of vasopressin. This shows that a lack of ADH was not the cause of diabetes insipidus in these patients, but that the impairment of concentrating ability must be located in the kidney.

According to present knowledge on concentrating mechanisms one has to distinguish between three different structures contributing to formation of a concentrated urine: the loop of Henle, which itself may be divided functionally into descending loop, thin ascending and thick ascending loop, the collecting duct, the vasa recta. Whereas both descending and thin ascending loop are thought to allow passive movement of water or ions, active sodium transport is only attributed to the thick ascending loop. This latter part represents the only active transport site within the concentrating system (22). The thin loop of Henle, the collecting duct and the vasa recta join to a countercurrent multiplier and exchange system. It is conceivable that in a partial renal diabetes insipidus, not all, but one or another of these structures might be affected. The 2 cases with medullary cystic disease might serve as an example of such a partial disturbance in concentrating power. Since medullary cystic disease seems to be a congenital malformation of the collecting ducts (21), one main exchange site concerning water and urea must be impaired. If the other concentrating mechanisms are assumed to be intact it is possible to expect some free water re-absorption though not an optimal concentrating ability. The patient with Bartter's Syndrome presents clinically with hypokalemia. Mannitius et al. (25) have demonstrated a decreased sodium concentration in the medulla of the kidney in hypokalemic dogs. These findings might imply that hypokalemia of Bartter's Syndrome impairs medullary sodium transport, i.e. either the passive in the thin ascending limb or the active in the thick ascending part or both. Partial renal diabetes insipidus was also observed in the patient with sickle-cell anemia. Hatch et al. (17) presume that sodium transport in sickle-cell disease is normal and the defect must be located in the vasa recta. Yet it still remains speculative whether the defect of the vasa recta is due to intravascular sickling and stasis or vascular occlusion (4, 38), or to an increase rather than a decrease in medullary blood flow secondary to an increased vascularisation demonstrated in these patients (12, 37). In any case the formation and maintenance of an adequate concentration gradient depends crucially on an adequate medullary blood flow which most likely is impaired in sickle-cell disease. Since the remaining structures of concentrating mechanism probably are functioning appropriately some re-absorption of free water should remain, leading to partial renal diabetes insipidus. The 4 patients of this group are, by nature of the disease, examples of partial renal concentrating defects. Other conditions such as hypo- and hypercalcemia, hypoproteinemia and hypothyroidism might also be expected to induce partial renal diabetes insipidus. Similarly it has been demonstrated by Aperia et al. (1) and Sommerschild et al. (33) that renal diabetes insipidus secondary to pyelonephritis might be present in very different degrees, corresponding to the degree of destruction of renal parenchyma in recurrent urinary tract infection.

resis might be attributable in a major part to a decrease of sodium concentration within the tubules as a consequence of the intensive osmotic diuresis (14 15 29 32) This could be due either to a washout of the medullary blood vessels (24 37) or to the increased speed and amount of fluid passing the tubules (23) Therefore it seems that TH_2O will depend on the kind of infusion used to provoke diuresis. In the presented studies sodium was supplemented to the mannitol infusion in amounts to maintain serum sodium level constant Water balance was maintained constant One can safely assume that mannitol induced depletion was therefore avoided.

It is not excluded nevertheless, that the mannitol induced overperfusion of the tubules influences the formation of the concentrating gradient in the medulla However in the present studies no TH_2O was observed

Many factors are known to influence the sodium re-absorption in Henle s loop and distal tubule thereby influencing the concentrating and diluting ability of the kidney (*a*) Delivery of sodium to Henle s loop depending on sodium balance on GFR (31) and on proteinemia (16) (*b*) Plasma potassium level (11 25) (*c*) Plasma calcium level (36) (*d*) Drugs with specific action on the concentrating sites (32) (*e*) Hormones such as thyroxine (19 35) (*f*) Extracellular fluid volume thought to be effective not only on the proximal tubule but also on Henle s loop and distal tubules (10 34) this, however not without objection (3) In the present studies serum sodium and potassium levels remained constant throughout the test Extracellular fluid volume expansion was very unlikely in these patients since much care was taken to maintain an equilibrated fluid balance during each individual study Proteinemia and calcemia were not controlled systematically during the study though during routine examinations of these patients proteinemia and calcemia were always found normal No clinical signs of hyper or hypothyroidism were present and in the cases with pituitary dwarfism determination of thyroxine has been normal. Thus the known factors influencing renal concentrating sites were comparably constant in all studies.

With the study procedure used it was possible to differentiate "normal patients from patients with diabetes insipidus, central or renal in origin Additionally patients with "partial diabetes insipidus were observed.

Normal patients (Table 2)

It seemed reasonable to assume normal concentrating ability in patients who had no clinical signs of renal disease normal glomerular filtration rate (GFR) and no signs of polyuria/polydipsia. One potomaniac was also included in this normal group. The observed TH_2O corresponds to the normal values of other authors (2, 15 33 39) Normal concentrating ability increases with increasing diuresis.

Partial central diabetes insipidus
(Table 3 group 1 a)

This group comprises 3 cases all able to re absorb free water under the stimulus of mannitol diuresis. However the rate of free water re-absorption (TH_2O) was below 1 S.D of what has been defined normal. When exogenous vasopressin was added all 3 patients were able to re-absorb more free water and their TH_2O was well within 1 S D of the normal value. Therefore it can be concluded that a normal medullary concentration gradient was established in these patients that the spontaneous antidiuretic activity however was less than optimal. This situation should be called "partial central diabetes insipidus" A similar observation has been described by Miller et al. (26) It is noteworthy that 2 patients suffered from central diabetes insipidus, but under treatment with carbamyl-dibenzo-azepine (Tegretol®) were able to concentrate their urine to some extent, although not optimally Studies of the action of carbamyl-dibenzo-azepine have favoured the assumption that this substance has an action on the hypo-

alamo-pituitary system in stimulating secretion of small amounts of ADH in some patients with central diabetes insipidus (13). It is conceivable that some antidiuretic activity was induced in the 2 patients but the amount was insufficient to allow for an adequate response to the stimulus of mannitol diuresis. Exogenously administered vasopressin was necessary for fully adequate response.

Partial renal diabetes insipidus
(Table 3 group 1 b)

The renal handling of free water in the 4 patients was similar to cases with renal diabetes insipidus, though some free water re-absorption was still possible. In contrast to the former group, their values for TH_2O did not increase despite administration of vasopressin. This shows that a lack of ADH was not the cause of diabetes insipidus in these patients, but that the impairment of concentrating ability must be located in the kidney

According to present knowledge on concentrating mechanisms one has to distinguish between three different structures contributing to formation of a concentrated urine: the loop of Henle, which itself may be divided functionally into descending loop, thin ascending and thick ascending loop, the collecting duct, the vasa recta. Whereas both descending and thin ascending loop are thought to allow passive movement of water or ions, active sodium transport is only attributed to the thick ascending loop. This latter part represents the only active transport site within the concentrating system (22). The thin loop of Henle, the collecting duct and the vasa recta join to a countercurrent multiplier and exchange system. It is conceivable that in a partial renal diabetes insipidus, not all, but one or another of these structures might be affected. The 2 cases with medullary cystic disease might serve as an example of such a partial disturbance in concentrating power. Since medullary cystic disease seems to be a congenital malformation of the collecting ducts (21), one main exchange site concerning water and urea must be impaired. If the other concentrating mechanisms are assumed to be intact it is possible to expect some free water re-absorption though not an optimal concentrating ability. The patient with Bartter's Syndrome presents clinically with hypokalemia. Mannitius et al. (25) have demonstrated a decreased sodium concentration in the medulla of the kidney in hypokalemic dogs. These findings might imply that hypokalemia of Bartter's Syndrome impairs medullary sodium transport, i.e. either the passive in the thin ascending limb or the active in the thick ascending part or both. Partial renal diabetes insipidus was also observed in the patient with sickle-cell anemia. Hatch et al. (17) presume that sodium transport in sickle-cell disease is normal and the defect must be located in the vasa recta. Yet it still remains speculative whether the defect of the vasa recta is due to intravascular sickling and stasis or vascular occlusion (4, 38) or to an increase rather than a decrease in medullary blood flow secondary to an increased vascularisation demonstrated in these patients (12, 37). In any case the formation and maintenance of an adequate concentration gradient depends crucially on an adequate medullary blood flow which most likely is impaired in sickle-cell disease. Since the remaining structures of concentrating mechanism probably are functioning appropriately some re-absorption of free water should remain, leading to partial renal diabetes insipidus. The 4 patients of this group are, by nature of the disease, examples of partial renal concentrating defects. Other conditions such as hypo- and hypercalcemia, hypoproteinemia and hypothyroidism might also be expected to induce partial renal diabetes insipidus. Similarly it has been demonstrated by Aperia et al. (1) and Sommerschild et al. (33) that renal diabetes insipidus secondary to pyelonephritis might be present in very different degrees, corresponding to the degree of destruction of renal parenchyma in recurrent urinary tract infection.

Diabetes insipidus (Table 3, groups 2 a and 2 b)

These patients present the well known conditions of central or of nephrogenic diabetes insipidus In patients affected with central diabetes insipidus (group 2 b) where the lack of ADH prevents a normal response to the hyperosmolar mannitol load a failure of re absorption of free water is seen in the first part of the study When however vasopressin is administered exogenously the kidney will re-absorb free water at a rate fitting the normality pattern Yet 3 patients of this group show a rather low value of free water re-absorption. This quantitative difference is interpreted as a wash-out effect of the medulla during a prolonged absence of endogenous vasopressin

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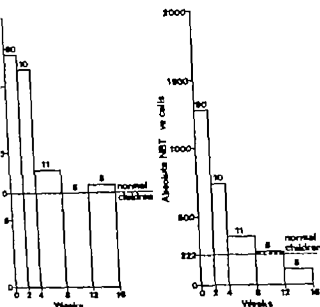


Fig. 1. Mean levels of NBT positive cells related to age after birth.

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Comparing newborns by gestational age it is found that pre term infants have lower levels of NBT positive cells than those with a longer gestation (>37 weeks). The results are statistically significant for absolute numbers of NBT positive cells (Table 2). Furthermore, the difference is even greater if the values for these infants are analysed on the basis of birth weight into those of very low birth weight (<2 000 g) compared to those of a higher birth weight (>2 000 g) as shown in Table 3

able 2. *Comparison by gestational age of results f NBT test on normal infants less than 14 days ld*

estational age	No.	NBT positive cells (Mean Range)	Absolute No of NBT positive cells Mean (Range)
37 weeks or less	27	15.5 (1–34)	712 (75–2 063)
		$p > 0.005$	$p < 0.01$
Greater than 37 weeks	63	18 0 (5–45)	1532 (34–7 443)

Table 3 *Comparison by birth weight of results of NBT test on normal newborns less than 14 days old*

Birth weight	No.	NBT positive cells Mean (Range)	Absolute No. of NBT positive cells Mean (Range)
<2 000 g	13	12.8 (1 30) $p > 0.10$	426 (75–1 200) $p < 0.001$
> 000 g	77	18 0 (4 46)	1423 (34–7 440)

DISCUSSION

The NBT test is of no value in the newborn period as an aid in the diagnosis of bacterial infection (5). Not only do 60% of newborns under 28 days old have high results but also a result in the normal range does not exclude bacterial infection. Cocchi et al. (1) have shown that "premature newborns" respond to bacterial infections by a fall in the numbers of NBT positive cells.

Acta Pædiat Scand 63 122–124 1974

LEUCOCYTE FUNCTION IN NORMAL AND PRE TERM INFANTS

K M GOEL and M R VOWELS

From the Department of Haematology Royal Hospital for Sick Children Yorkhill Glasgow Scotland

Recently there have been a number of reports indicating the usefulness of the nitro-blue tetrazolium (NBT) reduction test in the differentiation of bacterial infections from other febrile illnesses (3 6) However the results obtained in normal newborn infants are higher than those of older children (7) These high results overlap the range found in children with bacterial infection thus invalidating the test as a diagnostic aid in the newborn period Therefore the age at which results fall to the normal childhood range would determine the earliest age at which the test becomes of diagnostic value

The NBT test is also used as a screening test for chronic granulomatous disease (CGD). In affected children no NBT positive cells are found, absent dye reduction indicating poor bactericidal ability (4) Since other enzyme systems are known to have reduced activity in the pre term infant (e g glucuronyl transferase) we have also investigated the level of activity of the NBT test in this group of neonates.

MATERIALS AND METHODS

The NBT test was performed by a modification of the method of Park et al. (6). Capillary blood (0.5–1 0 ml) was collected into 50–100 units of heparin. Six drops of this blood were placed into a siliconised tube together with three drops each of 0 1% NBT dye and Krebs buffer (pH 7.2) containing 200 mg glucose per 100 ml. The mixture was incubated at 37°C for 15 min and then at room temperature for a further 15 min. Coverslip films were made and stained with Leishman s stain The percentage of polymorphs and monocytes containing a dark blue formazan deposit were counted. Total white cell and differential counts were performed on the same day

The groups tested consisted of 90 newborns aged less than 14 days, 31 infants aged 3–16 weeks and 19 control children aged 1–10 years. All were clinically normal with no evidence of infection.

Each group was selected on a random basis. All tests were read by the same observer Infants born before 37 weeks gestation and whose birth weight was 2 500 g or less were classified as pre-term. Small for dates infants have not been included in this group

Statistical analysis was performed by the Wilcoxon-Mann–Witney rank test (2)

RESULTS

Table 1 shows that infants over 5 weeks of age have similar numbers of NBT positive cells to normal children whereas newborns of less than 14 days have significantly higher results, both for percentage and absolute numbers.

Table 1 *Comparison by age of normal infants in children*

Age	No	NBT positive cells Mean (Range)	Absolute No of NBT positive cells Mean (Range)
Newborns 1–14 days	90	17.3 (2–46) $p < 0.001$	1 280 (60–7 440) $p < 0.001$
Infants 5–16 weeks	21	8 6 (2–14)	69 (26–1976)
Children 1–10 years	19	6.9 (1–17)	222 (15–530)

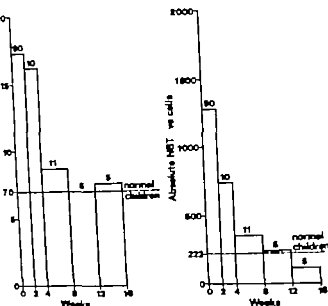


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A PILOT STUDY OF THE QUALITY OF HUMAN MILK IN A LOWER SOCIO-ECONOMIC GROUP IN KARACHI PAKISTAN

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A number of studies on the quality of human milk in lower socio-economic groups have shown a good fat, lactose and nitrogen content even under very primitive and poor conditions (10). A low protein content in the milk of women with a low protein intake has also, although more rarely been reported (5) This paper is a report of a pilot study of the total fat, lactose, total nitrogen, amino acid vitamin A and calcium content of milk collected from mothers who accompanied their infants to the Paediatric Clinic of Jinnah Postgraduate Medical Centre Karachi.

MATERIAL

The mothers came from the lower socio-economic group described earlier (7). They had all been breast feeding their babies up to the time of admission (see Table 1). In cases 2, 3, 8 and 9 breast milk had been the only food given, while the others had top-feeds of diluted buffalo milk. In 7 cases the cause of admission was diarrhoea and/or respiratory infection and in two cases diarrhoea and dehydration (cases 4 and 6). All the children displayed varying degrees of marasmic growth retardation. Pumping was performed 5 times in 24 hours at 3 hour intervals. One breast was emptied at every meal, both breasts at the last meal. The bottle was shaken thoroughly the amounts measured and 5 ml from every meal pooled in a deep-freeze. The rest was given to the baby by bottle or cup. Special care was taken to check that the mother did not feed her baby between meals. In most cases the baby was too weak for breast feeding and the collection of breast milk by pump was part of the necessary treatment. Because of this, co-operation with the mothers was good. The material was transported in the frozen state by one of us for further analysis in Stockholm, Sweden.

METHODS

All methods were standard methods, long in permanent use at the Research Department of the Milk Distribution Centre (AB Mjölkcentralen, Stockholm, Sweden). Fat was determined on 5 g of sample by solution in NaOH and determination according to Rose-Gottlieb. Lactose was then determined by the reduction method using Fehling's solution and total nitrogen according to Kjeldahl. Calcium levels were determined with the addition of ammonium oxalate and titration with potassium permanganate. Vitamin A was determined spectrophotometrically with activated glycerol-dichlorhydrin (14). The amino acid analysis was performed after acid hydrolysis (2 ml of diluted sample + 2 ml concentrated HCL, 72 hours at 105°C) on a system described in detail earlier (8). The levels of methionine-sulfoxide and methionine-sulfone were added to the methionine levels. Undetected losses of cystine and cysteine may have occurred, as they are unstable when hydrolysis is performed in the presence of carbohydrate. The material collected for this pilot study did not allow for oxidation and determination of cystine as cysteic acid (15). Loss of tyrosine can occur in the presence of carbohydrate and the tyrosine levels may be too low in this presentation (9). Tryptophan was determined after hydrolysis with BaOH and gel-chromatography by Dr Reynhold Kihlberg and Dr Björn Norrlind at the Department of Applied Microbiology of Karolinska Institutet (13).

RESULTS

The total fat, lactose, total nitrogen, vitamin A and calcium contents were, in this very poor group of urban women in an economically developing country not significantly

However our results show that after the fourth week of life the numbers of NBT positive cells are in the normal childhood range These findings are in agreement with those of Park et al. (7) who found that the numbers of NBT positive cells fall to normal childhood level 7–20 days after birth Therefore after the fourth week of life the NBT test may be useful as a diagnostic aid in bacterial infection

Comparing the results of the NBT test on newborn babies by birth weight we have found that those of birth weight <2 000 g have mean absolute numbers of NBT positive cells only one third that of newborns of birth weight >2 000 g (Table 3) A similar relationship may be seen in the data of Cocchi et al (1) although not commented upon by these authors. The relevance of this observation to susceptibility to infection in the new born period remains unclear

SUMMARY

The cytochemical nitroblue tetrazolium (NBT) reduction test was performed on 121 infants aged between 1 and 112 days. New born infants (normal and pre term) had higher levels of NBT positive cells than older infants and children These high levels fell to the normal childhood range after the fourth week of life Infants aged less than 14 days with a birth weight of <2 000 g had a mean level of NBT positive cells one third that of infants of higher birth weight (>2 000 g)

ACKNOWLEDGEMENT

The authors are grateful to Dr I Dunmore for performing the statistical analysis.

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Key words. Nitroblue tetrazolium reduction test, chronic granulomatous disease glucuronyl transferase, Krebs buffer pre-term infants

Table 3 *Protein content*

Mean and ±2 S.E. are given

(a) Total amino acids = *1.01 ± 0.13 g/100 ml*
Compare 1) Souport, Brussels (15). *1.01 g/100 ml*
2) FAO Rome, 1970 (1) *1.05 g/100 ml*

(b) Amino acid residues = *0.82 ± 0.11 g/100 ml* (true protein)
Compare 1) Souport, Brussels (15): *0.83 g/100 ml*
2) Saito, Tokyo (11) *0.81 ± 0.11 g/100 ml*

(c) Total nitrogen = 185 ± 20 mg/100 ml
N from amino acids and NH_4 = 165 ± 18 mg/100 ml = 89 % of Total N
Counting 7 10 % of N from urea (15), protein content = (80/100) 0 185 6.25 = *0.9 g/100 ml*
Compare 1) Souport, Brussels (15):
Nitrogen from amino acids and NH_4 = 88 % of Total N
Protein content = (80/100) 0.173 6 25 = *0.9 g/100 ml*
2) Hytten, Aberdeen (3):
Total N 196 *mg/100 ml*, NPN 53 *mg/100 ml (over 6 weeks lactation)*
Protein content = (0.196–0 053) 6.25 = *0.9 g/100 ml*

Thus: true protein content = normal = *0.8–0.9 g/100 ml*

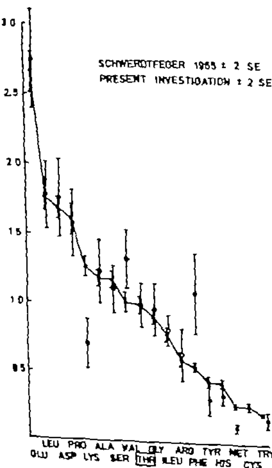


Fig 1 The molar relationships of the various amino acids of normal human milk (•—•) (12), and of the present investigation ( ). The concentration of threonine has been taken as the unit = 1.0.

of the child s illness and hospitalization, and for the family members at home.

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A diet such as the general South Asian diet, consisting mainly of rice or wheat, is deficient in lysine. In the absence of supplementation by milk in low socio-economic groups, a diet of mainly cereals and pulses ("*dal*") will also be deficient in methionine. The results of this study suggest that this dietary deficiency is reflected in the milk produced by these women By way of comparison, the protein composition of cow milk may vary on a nutritional basis (6 8). The unchanged nitrogen level, and the pattern of decreased levels of

Table 1 *Material*

Mothers	Age	Weight (kg)	Height (cm)	Pregnancy number	Surviving children	Family income Rs/month
1	23	41	145	3	2	300
2	20	36	147	2	2	170
3	16	54	153	2	1	100
4	43	54	150	10	10	120 (nil 6 months)
5	7	63	161	5	4	150
6	26	40	138	5	3	100
7	21	32	145	6	6	120
8	20	44	150	4	4	250
9	30	36	147	1	1	100 (nil 6 months)

Infants	Age (months)	Admission weight (g)	Duration of illness (days)
1	4	6 300	15
2	6	3 600	8
3	4	4 050	60
4	1.5	4 300	40
5	3.5	5 400	6
6	4	2 700	15
7	4	5 400	7
8	4	4 950	7
9	6	2 700	10

lower than has been found in more affluent countries (Table 2)

The protein content counted as the sum of amino acid residues, was 0 82±0 11 g/100 ml and consistent with the findings in Brussels (15) and Tokyo (11) (Table 3). Protein content, calculated from nitrogen content was in complete agreement with the results from Brussels (15) and Aberdeen (3). The amino acid analysis showed very low levels of lysine and methionine while the valine and phenylalanine levels were higher than those of available normal materials (Fig 1)

DISCUSSION

The milk volume which is the most important variable behind failures in breast milk feeding was low and probably greatly influenced by the mother s anxiety on account

Table 2 *Total fat lactose total nitrogen vitamin A and calcium content*

Sample	Fat (g/100 ml)	Lactose (g/100 ml)	N (mg/100 ml)	Vit. A (IU/100 ml)	Ca (mg/100 ml)
1	1 70	7 31	132	208	22.9
2	5 72	6.47	175	185	20.6
3	1 80	5.57	208	139	23 0
4	2.28	5 79	203	149	33.3
5	2.23	7 19	166	55	32.6
6	2.81	5.20	159	150	30 7
7	2.80	6.88	208	125	34 0
8	2.21	6.90	232	322	40.2
9	3 06	4.51	177	116	18 6
Mean	2.73	6.20	185	161	28 4
S.D	1.21	0.98	31	74	7 4
S E.	0.40	0.33	10	25	2.5
Normal	3.36	6.90	196	190 (2)	34 (2)
>6 weeks					
lactation	0.91	0 18	22		
(3)	0.24	0.05	6		

Table 3 *Protein content*

Mean and ±2 S.E. are given

(a) Total amino acids = *1.01 ± 0.13 g/100 ml*
Compare 1) Souphart, Brussels (15): *1.01 g/100 ml*
2) FAO Rome, 1970 (1) *1.05 g/100 ml*

(b) Amino acid residues = *0.82 ± 0.11 g/100 ml* (true protein)
Compare 1) Souphart, Brussels (15): *0.85 g/100 ml*
2) Saito, Tokyo (11) *0.81 ± 0.11 g/100 ml*

(c) Total nitrogen = 185 ± 20 mg/100 ml
N from amino acids and NH_4 = 165 ± 18 mg/100 ml = 89 % of Total N
Counting 7–10 % of N from urea (15), protein content = (80/100) 0.185 6.25 = *0.9 g/100 ml*
Compare 1) Souphart, Brussels (15):
Nitrogen from amino acids and NH = 88 % of Total N
Protein content = (80/100) 0.173 6.25 = *0.9 g/100 ml*
2) Hytten, Aberdeen (3):
Total N 196 mg/100 ml, NPN 53 mg/100 ml (over 6 weeks lactation).
Protein content = (0.196–0.053) 6.25 = *0.9 g/100 ml*

Thus: true protein content = normal = *0.8–0.9 g/100 ml*

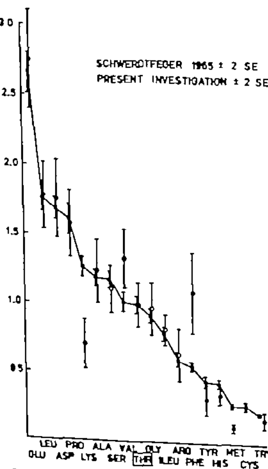


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methionine and lysine in combination with an increased valine level and an increased phenylalanine/tyrosine ratio suggest an increased level of caseins and a decreased level of lactalbumin (4) A higher level of caseins than in Western European materials has been reported from Japan (11) These findings indicate the necessity of further examination of the protein composition of human milk its possible genetic variation as well as its dependence on maternal diet and maternal nutritional status

SUMMARY AND CONCLUSION

The total fat lactose total nitrogen protein vitamin A and calcium content of human milk from a very low socio-economic group in an economically developing country corresponded to known normal levels. The amino acid content however showed a comparative decrease in the content of two essential amino acids (lysine and methionine) This could imply that the nutritional value of the milk protein is substantially reduced

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A FOLLOW-UP STUDY OF CHILDREN WITH ASTHMATOID BRONCHITIS

II. *Serum IgE and Eosinophil Counts in Relation to Clinical Course*

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From the Department of Paediatrics and the Blood Centre University Hospital Uppsala, Sweden

In children with asthmatoid bronchitis a minority will develop asthma (5 8). Since it is important to treat asthma as early as possible, it is of importance to distinguish those children with asthmatoid bronchitis who are at risk of developing asthma. For such children, preventive measures should be taken to try to reduce the likelihood of development of hypersensitivity to different allergens. It may however be difficult in the single case to estimate the risk for subsequent asthma. Special regard is usually paid to the presence or absence of factors that are considered to be combined with a high risk for subsequent asthma. Such factors are allergy in the family atopic dermatitis in the child, a late age of onset of wheezing, recurrent symptoms and eosinophilia in blood or secretions (5 8, 11 22).

As the serum IgE concentration has been found to be increased above the 95th percentile in up to about 60% of children with asthma (4), determination of the serum IgE concentration might be of help in assessing the prognosis in children with asthmatoid bronchitis (9 21). The aim of the present study was to investigate the value of serum IgE determinations and blood eosinophil counts as prognostic tools in children with asthmatoid bronchitis and to study longitudinally the IgE levels in relation to the clinical course.

MATERIAL AND METHODS

For a presentation of the patient material and the radioallergosorbent test (RAST) technique and allergen test panel in RAST the reader is referred to a previous paper (10).

Definitions of patient groups. At the end of the investigation period the 81 children in the study were divided into four groups according to the clinical history. The following definitions of the groups were used. *Group 1 (asthma)*: At least one attack of apparently exogenously provoked asthma or at least two attacks of wheezing with no apparent connection with a respiratory tract infection. *Group 2 ("asthmatoid bronchitis")*: Wheezing only in connection with respiratory tract infections and with one or more such attacks during the last year. *Group 3 (other allergy)*: Atopic eczema or at least one attack of urticaria or at least two attacks of allergic rhinoconjunctivitis without any evidence of asthma. *Group 4 ("healthy")*: Children with wheezing episodes only in connection with respiratory tract infections and with no such symptoms during the last year

Normal material for IgE determinations

Blood specimens were taken from 57 healthy children between 2 and 10 years of age selected at random by the Central Bureau of Statistics, Stockholm. Sera from these children were also used for studies previously reported on immunoglobulin levels (3). Furthermore, 51 healthy children below 2 years of age were selected at random from Child Health Centers in Uppsala and 9 newborns from the Department of Obstetrics, University Hospital, Uppsala. The children had never demonstrated any allergic symptoms. Venous blood was drawn from the children above 2 years of age and capillary blood from the younger and also from few older children in whom venepuncture failed. From the newborns blood was

methionine and lysine in combination with an increased valine level and an increased phenylalanine/tyrosine ratio, suggest an increased level of caseins and a decreased level of lactalbumin (4) A higher level of caseins than in Western European materials has been reported from Japan (11) These findings indicate the necessity of further examination of the protein composition of human milk its possible genetic variation as well as its dependence on maternal diet and maternal nutritional status.

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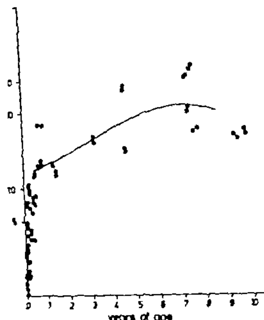


Fig. 2 The serum IgE concentrations in 117 healthy children without any allergic history. The superimposed regression function is based on the IgE values of the age interval 1/2 10 years to obtain optimal adaptation to the normal values at ages relevant for the patients under study.

Table I *The mean serum IgE concentrations and the 95% confidence limits in 117 healthy children*

Age groups	*n*	Mean (units/ml)	95% confidence limits
Newborns	9	1.6	0.7 3.4
6 weeks–3 months	24	4.4	1.1–17.2
3 months 9 months	14	12.9	2.6–65.2
9 months–2 years	13	18.4	6.4–53.0
2 years–4 years	18	27.0	7.1–103.3
4 years–6 years	15	42.9	7.6–242.4
6 years–10 years	24	53.3	7.8–391.6

The mean number of eosinophil cells per mm^3 blood was also highest in *group* 1 and significantly higher than in group 2 ($p < 0.001$) and *group* 3 ($p < 0.05$). The mean eosinophil totals in groups 2 and 3 differed significantly from that of the "healthy" group ($p < 0.001$). The Table also shows that in comparison with the "healthy" group, (i) allergy in the family was about twice as common in the "non healthy" groups; (ii) the age of onset was higher in the asthma *group but lower in the* group with other allergy (iii) the children considered to have asthma were somewhat older than the others (iv) the number of *wheezing attacks* in connection with respiratory tract infections during the investigation period was about 2–4 times higher in the "non-healthy" groups with the highest mean in the asthma group. Six of the children considered as "healthy" had suffered from 5–6 attacks *of* asthmatoid bronchitis during the observation period. These children did *not* have *higher* eosinophil counts or higher IgE concentrations than "healthy" children with only a few attacks.

Although the mean IgE concentration and the mean eosinophil totals were higher in the "non-healthy" *groups than in the "healthy"* group there was a considerable variation *among patients in all groups* as regards the number of samples with high values (Table 3). The majority of the children in groups 1 and 3 had high serum IgE in at least one sample while the frequency was 42% (8/19) in group 2 and 26% (9/35) in group 4. If a child was found to have high IgE in *one* sample there was a high probability of finding high IgE values also in the following serum samples. Of the children with high IgE in at least one sample, 40–60% had high levels in at least 75% of the samples regardless of the clinical diagnosis. Single values in *group 4 ("healthy")* were almost as high as the highest in group 1 (asthma).

Of the 19 children in group 1 with eosinophilia in at least one sample, 6 children had eosinophilia in more than 75% of the samples (Table 3).

Only one more child had such a *high frequency* of samples with eosinophilia. This child had also high serum IgE in most of the samples but clinically she was regarded as "healthy". *High-grade* eosinophilia (>700 cells/mm^3) was only exceptionally seen in more than 50% of the samples from one and the same child and then only in the groups 1 and 2.

As seen from Table 4 a high serum IgE con-

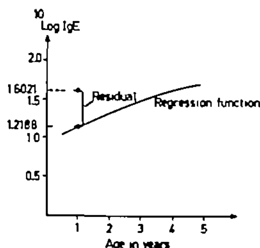


Fig 1 Principle for calculation of residuals. Se example in the text

taken from the umbilical cord After clotting, serum was separated from the samples and stored at −20°C.

The serum IgE concentration was determined by the radio-immunosorbent technique (RIST) (24) in the modification for IgE estimation described previously (14) In this study RIST was used as a sequence test having the following principle: One ml of Sephadex coupled rabbit anti IgE (Phadebas IgE test, Pharmacia Ltd Uppsala, Sweden), 33 μg/ml was incubated with 0.2 ml of the patient's sera diluted 1 10 and 1 100 After incubation at room temperature over night, 0 1 ml ^{125}I labelled IgE, corresponding to about 25 000 counts per minute was added. After another incubation period for 3–4 hours, the tubes were washed five times with saline containing 0 1% Tween 20 The remaining radioactivity on the particles was thereafter measured in a gamma scintillation counter All sera obtained from a child were tested at the same time, in duplicate and at two different occasions. The error of the method calculated as the mean deviation in duplicate analysis of the samples was about 8% The results were expressed in units/ml (WHO standard 68/341) which are about 1/2 of the values expressed as ng/mL *The serum concentrations of IgG IgA and IgM* were measured by single radial immunodiffusion in agar gel according to the method of Mancini et al., with minor modifications (13)

Blood eosinophil counts were performed as a routine test in a *Fuchs Rosenthal counting chamber* The mean number of eosinophil counts was 6 8 per child. Eosinophilia was defined as more than 400 eosinophils/mm³ and high-grade eosinophilia as more than 700 cells/mm³ This latter value approximately corresponds to the mean value +2 S.D in healthy children (7 20)

Statistics

Because of the strong correlation between age and IgE level it was necessary to relate every single IgE value of a patient to the age at the time of sampling. This has been solved by comparing every observed IgE value with a normal value calculated from a regression function. *In attempting to find a function* that can give normal values of serum IgE in the actual interval of age, a third degree polynomial based on the values of the normal children at ages from 1/2 to 10 years was found to be the most useful.

The regression function chosen was the following

$$^{10}\log IgE = 1\ 1460 + 0\ 0536\ X + 0\ 02159\ X^2 - 0.00238\ X^3 \quad (1)$$

X = age in years
R = 0.5037 (the multiple correlation coefficient)
S = 0.3457 (the standard deviation of residuals)

An IgE value of 40 units/ml in a 1 year-old child is calculated in the following way

$^{10}\log 40 = 1\ 6021$

The normal value of $^{10}\log$IgE in a 1 year-old child, 1.2188 is calculated by substituting the value 1 for X in (1) The difference between the observed value and the normal value is 1.6021 − 1.2188 = 0.3822. This value is called the residual. Fig. 1 shows the regression function the IgE value used in the example and the residual

To facilitate the judging of residuals calculated as described above, zeta" (*z*) values have been introduced. These values can be considered standardized residuals and are calculated by dividing the residual by the standard deviation of residuals mentioned above (S). In this way it is possible to express a deviation from a normal value as a number of standard deviations ( ). In the example given above the value is 0.3833 0.3457 = 1 1088 The values were used for all comparisons between different groups of patients.

RESULTS

The serum IgE concentrations in healthy children without any history of allergic symptoms are seen in Fig. 2. The concentrations in umbilical cord sera were very low but during the first year of life a rapid increase was seen. After that age a slower increase was observed. The mean IgE concentrations and the 95% confidence limits for different age groups are given in Table 1

Table 2 shows that compared with group 4 ("healthy") the mean IgE concentration was significantly higher in all "non healthy" groups ($p < 0\ 025 - < 0\ 001$) The highest mean IgE value was found in group 1 (asthma) and this value was significantly higher than that of group 2 ($p < 0\ 05$) but not than that of group 3

Table 4 *The number of children with high serum IgE and blood eosinophilia in the different patient groups*

	Group 1 (asthma) (n = 19) n (%)	Group 2 ("asthmatoid bronchitis") (n = 19) n (%)	Group 3 (other allergy) (n = 8) n (%)	Group 4 ("healthy") (n = 35) n (%)
In the last blood sample				
IgE > mean + 1 S.D	12 (63)	8 (42)	6 (75)	6 (17)
IgE > mean + 2 S.D	9 (47)	6 (32)	3 (38)	4 (11)
Eosinophils > 400/mm³	8 (42)	7 (37)	1 (13)	9 (26)
Eosinophils > 700/mm³	2 (11)	2 (11)	1 (13)	2 (6)
In at least one blood sample				
IgE > mean + 1 S.D	14 (74)	14 (74)	7 (88)	14 (40)
IgE > mean + 2 S.D.	13 (68)	8 (42)	5 (63)	9 (26)
Eosinophils > 400/mm³	19 (100)	15 (79)	5 (63)	16 (46)
Eosinophils > 700/mm³	17 (89)	11 (58)	5 (63)	5 (14)

in group 1 than in group 4. In all four groups the lowest z values were found in the beginning of the year and the highest in the autumn (Fig. 3). This variation with time was most pronounced in groups 2–4 and barely noticed in group 1. The pattern was more pronounced in some children but none of these had a known pollen allergy. A variation of the serum IgE concentration along with the pollen season was seen only during 2 of 3 seasons in 1 out of 4 children with pollen allergy verified by positive skin tests, RAST and provocation tests. The serum concentrations of immunoglobulins G, A and M did not show any consistent variation with the time of the year in 8 children with pronounced seasonal variation of IgE.

Fig. 4 shows the children with a pronounced rise in IgE level during the follow-up period. Most of these children seemed to improve with time. Wheezing during respiratory tract infections became less frequent and less severe. In 4 children a definite fall of the IgE concentration was seen with time (Fig. 4). Two of the children had asthma, one urticaria and one "asthmatoid bronchitis". The decrease in IgE went hand in hand with a clinical improvement in all 4 children.

In some children changes in the serum IgE levels paralleled the changes in allergen-specific IgE as measured by RAST. Such parallelism was mainly seen in children with IgE antibodies only to one of the 10 allergens in the test panel (10) (Fig. 5). When IgE antibodies were found to more allergens the agreement between IgE and allergen-specific IgE was less

Table 5 *Laboratory data during the first month of study in 53 first time-wheezers*

Patient groups	n	IgE > mean + 2 S.D n (%)	Eosinophils > 400/mm³ n (%)	Eosinophils > 700/mm³ n (%)	High IgE + eosinophils > 400/mm³ n (%)	High IgE + eosinophils > 700/mm³ n (%)
Group 1 (asthma)	9	4 (44)	8 (89)	4 (44)	4 (44)	2 (22)
Group 2 ("asthmatoid bronchitis")	10	1 (10)	3 (30)	1 (10)	0	0
Group 3 (other allergy)	7	3 (43)	4 (57)	3 (43)	3 (43)	2 (22)
Group 4 ("healthy")	27	2 (7)	5 (19)	1 (4)	1 (4)	0

Table 2. *Laboratory and clinical data during the follow up of 81 children with asthmatoid bronchiti*

Patient groups	n	Serum IgE as mean value[a]	Mean no eosinophils/ mm³	Allergy in parents and/or siblings	Mean age of onset (months)	Mean age at end of follow-up (months)	Mean no wheezing attacks[b]
Group 1 (asthma)	19	1.9104	535	10 (53 %)	13 6	54 6	8.2
Group 2 ("asthmatoid bronchitis")	19	0 9053	345	8 (44 %)	11.3	44 0	6.6
Group 3 (other allergy)	8	1 1935	411	4 (50)	8.1	40.6	4.3
Group 4 ("healthy")	35	−0 0451	230	8 (23 %)	10.4	41.9	2.3

[a] The value is the number of S D in relation to the normal distribution at corresponding ages.
[b] In infected children during the follow-up period.

centration in the last serum sample was found about 4 times more often in groups 1 and 3 than in group 4 both when the limit for a high IgE was the mean value +1 S D and +2 S D respectively The frequency of children with high IgE in group 2 was 2–3 times higher than that of group 4 When the calculations were based on the occurrence of high IgE in any of the samples, the number of children with high IgE increased in all groups but most in group 4

When judged only from the last blood sample there was a greater difference between "healthy and "non healthy children as regards the percentage with high IgE compare with the percentage with eosinophilia (Tabl 4) When a high value in any of the sample was counted high IgE and eosinophilia (>400 mm³) discriminated between "healthy" an "non healthy children to the same extent However high-grade eosinophilia discriminate better than a high IgE value between the sam groups.

Fluctuations of the IgE concentrations (expressed as z values) from one sampling tim to another were seen in all children. Such fluctuations seemed to be more pronounce

Table 3 *The frequency of samples with either serum IgE concentrations in excess of 2 S D abo the mean value or eosinophilia in patients in the different groups*

Patient group	n		Number of children in whom the percentage of samples with high IgE and/or eosinophilia was: 0	>0–25	25–75	>75
Group 1 (asthma)	19	High IgE	6	2	3	8
		Eos >400/mm³	0	4	9	6
		Eos >700/mm³	2	8	8	1
Group 2 ("asthmatoid bronchitis")	19	High IgE	11	2	2	4
		Eos >400/mm³	4	1	14	0
		Eos >700/mm³	8	7	4	0
Group 3 (other allergy)	8	High IgE	3	0	3	2
		Eos >400/mm³	3	0	5	0
		Eos >700/mm³	3	3	2	0
Group 4 ("healthy")	35	High IgE	26	4	0	5
		Eos >400/mm³	19	2	13	1
		Eos >700/mm³	30	3	2	0

2.5%). A high-grade eosinophilia (>700/mm³) alone had the same negative prognostic value as the combination of a high IgE value + eosinophilia (>400/mm³).

DISCUSSION

The results of the IgE determinations in normal children are in good agreement with those previously reported from this laboratory (3 15) and from other laboratories (2, 23) when special regard is paid to the different units used to express the concentrations. There is one difference however during the first months of life the IgE concentrations are much lower in this series than previously reported from this laboratory The main reason for this is the slight modification of the RIST technique introduced, which includes a pre-incubation of the samples from patient as well as standard with the particle-bound anti-IgE before the labelled IgE is added. An increased sensitivity of the test is then obtained which allows the patients sera to be tested at higher dilutions. When sera are tested undiluted, the high concentration of protein or specific inhibitors seem

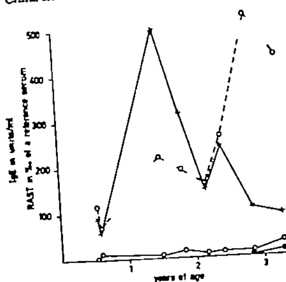


Fig 6 Serum IgE (O--O) and IgE specific to horse dander (●—●), house dust (O—O) and egg white (—×) in a child with manifest urticaria during follow-up.

to interfere with the binding of IgE on the particle-bound anti-IgE. As the uptake of labelled IgE then becomes falsely low the patient IgE will be interpreted as higher than it really is (16).

In this study children considered to have asthma had significantly higher serum IgE concentration than healthy children. The results are in agreement with other reports (4 12, 18 23 25) and confirm that childhood asthma often is mediated by antibodies of the IgE class. The frequency of high IgE levels in childhood asthma varies considerably however and figures between 26% (18) and 56% (4) have been reported. This discrepancy may probably be explained by heterogeneous patient materials. In the present study 47% of the children considered to have asthma had a high IgE level in the last serum sample at a time when the clinical diagnosis was settled. As the IgE concentrations could vary from one test occasion to another the number of children with high IgE levels increased with the number of tested samples. Thus, 74% of the asthmatic children had a high serum IgE concentration in at least one serum sample. Also in the othe

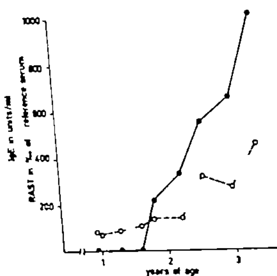


Fig 5 Serum IgE (O -O) and IgE specific to horse dander (●—●) in a child with the clinical diagnosis "asthmatoid bronchitis"

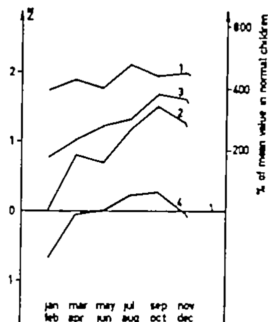


Fig 3 The mean z values for serum IgE in the different patient groups in relation to the time of the year The results are based on all values obtained during the whole investigation period. The broken line is drawn down to the Jan-Feb IgE level to emphasize the cyclic course. z = 1 corresponds to $10^{0.3457} \approx 2.22$ (0.3457 = 1 S D of the residuals).

good (Fig 6) IgE antibodies to animal danders, pollens or house dust were found only in children with IgE concentrations greater than 2 S D over the mean in all or almost all of the serum samples. The only exception was a boy with a normal IgE but with a low titre of IgE antibodies to cat dander Bronchial provocation test with the cat dander extract 10^{-1} w/v was negative

IgE antibodies to egg white or cow s milk were found in many children with normal IgE but high titres were found only in children with high IgE.

Three children had had mild atopic eczema before they were included in the study All three had high IgE concentrations in almost all serum samples and two of the children were considered to have asthma and one urticaria.

Recurrent wheezing was combined with an increased risk for subsequent asthma. Among the 28 children who had suffered from one or more attacks of "asthmatoid bronchitis before they were included in the study 36% were considered to have asthma and 29% to be "healthy" at the end of the follow-up. Among the 53 children wheezing for the first time when the study began, 17% developed asthma and 51% became "healthy Therefore only first time wheezers were considered suitable for testing the value of the serum IgE concentration as a prognostic tool. Table 5 shows that scarcely 50% in the groups 1 and 3 had an increased serum IgE concentration in one or both of the two first serum samples, the first taken during the acute wheezing period, the second at a control 3 weeks later The frequency of children with high IgE in the groups 2 and 4 was considerably lower Of the 10 children with high IgE in the first samples only 2 (20%) were considered as healthy at the end of the follow-up Of the children with initial eosinophilia (> 400/mm³) 25% became "healthy and if eosinophilia as well as a high IgE value was found the frequency of "healthy children was reduced to 1 in 8

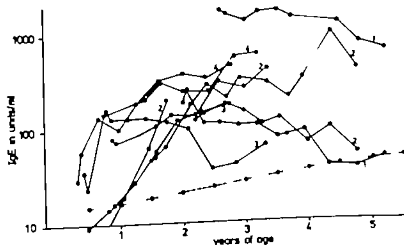


Fig 4 The serum IgE concentrations in relation to age in 10 children with increase (●) or decrease (○) of IgE during the observation period. The diagnosis group number is given for each child The broken line represents the mean serum IgE in healthy children

(12.5%). A high-grade eosinophilia (>700/mm³) alone had the same negative prognostic value as the combination of a high IgE value + eosinophilia (>400/mm³).

DISCUSSION

The results of the IgE determinations in normal children are in good agreement with those previously reported from this laboratory (3, 15) and from other laboratories (2, 23) when special regard is paid to the different units used to express the concentrations. There is one difference however: during the first months of life the IgE concentrations are much lower in this series than previously reported from this laboratory. The main reason for this is the slight modification of the RIST technique introduced, which includes a pre-incubation of the samples from patient as well as standard with the particle-bound anti-IgE before the labelled IgE is added. An increased sensitivity of the test is then obtained which allows the patients sera to be tested at higher dilutions. When sera are tested undiluted, the high concentration of protein or specific inhibitors seem to interfere with the binding of IgE on the particle-bound anti-IgE. As the uptake of labelled IgE then becomes falsely low, the patient IgE will be interpreted as higher than it really is (16).

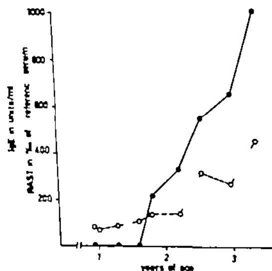


Fig. 5. Serum IgE (O- -O) and IgE specific to horse dander (●—●) in a child with the clinical diagnosis asthmatoid bronchitis.

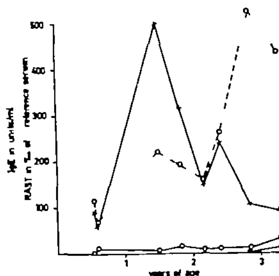


Fig. 6. Serum IgE (O- -O) and IgE specific to horse dander (●—●), house dust (O—O) and egg white (—×) in a child with manifest urticaria during follow-up.

In this study children considered to have asthma had significantly higher serum IgE concentration than healthy children. The results are in agreement with other reports (4, 12, 18, 23, 25) and confirm that childhood asthma often is mediated by antibodies of the IgE class. The frequency of high IgE levels in childhood asthma varies considerably however and figures between 26% (18) and 56% (4) have been reported. This discrepancy may probably be explained by heterogeneous patient materials. In the present study 47% of the children considered to have asthma had a high IgE level in the last serum sample at a time when the clinical diagnosis was settled. As the IgE concentrations could vary from one test occasion to another, the number of children with high IgE levels increased with the number of tested samples. Thus, 74% of the asthmatic children had a high serum IgE concentration in at least one serum sample. Also in the other

groups an increased number of children with high IgE levels was seen and this increase was greatest in the "healthy" group That no less than 26% of the children in this group had a high serum IgE level in at least one serum sample may indicate that some of these children are potential asthmatics. In fact three of the children considered as healthy had high IgE concentrations as well as high grade eosinophilia on most test occasions and three more children on a few occasions. There was no evidence of parasitic infestations in these children. Two of the children had antibodies of the IgE class to egg white one of them at a high titre Another child had a low concentration of IgE antibodies to cat dander As they had shown no apparent asthma or other allergic manifestations during the investigation period they were classified as "healthy" but they probably must be regarded as children at very high risk for manifest allergy

The high serum IgE concentrations in many children in the group of "asthmatoid bronchitis" may indicate that some of them in fact have reaginic asthma though not clinically recognized Six of the 8 children with high IgE in this group had high-grade eosinophilia in at least one sample and in 2 of these children IgE antibodies were found to animal danders and in one of them also to pollen Bronchial provocation tests in these 2 children were positive.

Blood eosinophilia was most common in the asthma group but high IgE levels were almost as common in group 3 (other allergy) as in the asthma group High serum IgE concentrations are reported not to be as frequent in children with urticaria and allergic rhinoconjunctivitis as in children with asthma (4 17) This suggests that in this study also some of the children in group 3 are asthmatics.

Repeated attacks of asthmatoid bronchitis makes the diagnosis asthma likely and some authors diagnose asthma after three or more attacks of wheezing in infected children (6 22) The number of wheezing attacks related to respiratory tract infections during the observation period was surprisingly high in all groups. The high frequency of wheezing attacks may be explained by the close personal contact with the patients which may have lead to the reporting of more mild or moderate attacks than would have been remembered and reported at, for example yearly examinations. The children with asthma had the highest mean number of wheezing episodes during infections but the individual variations were great in all groups. In the "healthy" group up to six attacks were noted and these children did not have higher IgE concentrations or higher eosinophil counts than the other children in the "healthy" group. However it may be that these children who had several attacks are more liable than the others to develop symptoms of asthma or bronchitis in the future

In some children the IgE concentrations showed a definite increase or decrease during the follow up period Such changes were clear cut in only a minority of the children A decrease in the IgE concentration with time was seen in a few children considered to have asthma urticaria or asthmatoid bronchitis. This decrease was accompanied by a clinical improvement which probably indicates that the children suffered from reaginic allergy and that the decrease of IgE reflects a spontaneous improvement or cure of the disease Similarly the children with IgE values increasing above the normal might be expected to develop asthma or other kinds of reaginic allergy Up to now one of these children has had urticaria but none has yet clinically manifest asthma. However one of the children with asthmatoid bronchitis has immunologically verified asthma with an increasing titre of IgE antibodies to horse dander (Fig. 5) and a positive bronchial provocation test and another child has a low titre of IgE antibodies to cat dander but a negative bronchial provocation test In spite of the decreasing tendency to wheeze during respiratory tract infections it is thus in some of the children with increasing IgE apparently a reaginic allergy in development

In patients with atopic dermatitis, Öhman reported a seasonal variation of the IgE concentration with the lowest values during the summer when the dermatitis was least severe (26). Kumar et al. (19) did not find any obvious variation in the IgE concentration with the time of the year in samples taken at monthly intervals from 26 children with asthma. In the present study results similar to those of Kumar were obtained for the children with asthma. It was therefore surprising to find marked seasonal variation in the IgE levels in children in the other groups. Fluctuations of the IgE levels in serially taken samples were seen in patients in all groups and of course such fluctuations may accidentally simulate a seasonal variation in some cases. The consistency of the pattern seen in a more or less pronounced form in all groups makes such an explanation unlikely. A stimulation of the IgE synthesis by common pollen allergens is less probable as the rise started in late winter and no IgE antibodies to pollen allergens were found in RAST in the children with the most pronounced seasonal variation in their IgE. Other allergens such as moulds are more suspect as the long stimulation period is more characteristic for moulds than for pollens. Respiratory tract infections, the great majority probably of viral origin, did not seem to raise the IgE level but as such infections are common in children, samplings 3–4 times in a year is not sufficient to enable evaluation of a possible stimulating effect. The explanation for the barely observed seasonal variation of IgE in the asthma group may be that the IgE synthesis in this group to a great extent is influenced by allergens and the childrens contacts with these are not necessarily seasonally dependent.

Further longitudinal studies of IgE in healthy and allergic individuals are needed to evaluate and define the causes of a possible seasonal variation.

One of the main purposes of this study was to investigate the prognostic value of a serum IgE determination in a child with its first attack of asthmatoid bronchitis. The results suggest that a high IgE concentration indicates asthma better than does a moderate eosinophilia. However, asthma seemed to be predicted better by a high-grade eosinophilia than by a high IgE value. An increased number of eosinophils may be seen in many kinds of disorders and it is seen in "extrinsic" as well as in "intrinsic" asthma (1). An increased serum IgE concentration is almost exclusively found in extrinsic asthma, in other atopic disorders and in parasitic infections (15). The combination of a high IgE concentration and eosinophilia in a wheezing child will therefore strongly suggest that the child suffers from reaginic asthma.

Although only 44% of the children wheezing for the first time and who would manifest asthma within the next 3 years had a high serum IgE concentration initially, determination of the IgE concentration appears to have a definite value in the clinical assessment of the prognosis. A high serum IgE concentration is highly indicative of subsequent asthma especially when found in repeated samples. Together with clinical data the IgE concentration and the eosinophil count will give a comparatively high degree of certainty in the assessment of the possible allergic component in wheezing children. Furthermore, the results suggest that screening for IgE antibodies to common allergens as animal danders, pollens or house dust is hardly worth while at these ages if the serum IgE concentration is normal.

SUMMARY

After a follow-up period for 15–40 months (mean 31 months) of 81 children, aged 2–66 months, with asthmatoid bronchitis 19 (23%) were clinically considered to suffer from true asthma and 8 (10%) from allergic rhinoconjunctivitis or urticaria. These children had significantly higher serum IgE concentrations and a significantly greater number of blood eosinophils than the 35 children considered to be "healthy". Nineteen children (23%) had still

episodes of wheezy bronchitis at the end of the investigation period and these children also had significantly higher serum IgE levels and a significantly greater number of blood eosinophils than the "healthy children Although none of the children with "asthmatoid bronchitis had clinically apparent asthma 2 had IgE antibodies to animal danders and pollen as shown by RAST and in both bronchial provocation tests were positive Another 4 children in this group had high IgE as well as an increased total of blood eosinophils (>700/mm³) indicating that they probably had reaginic asthma

A high serum IgE concentration was found initially in 44 of first-time-wheezers who became asthmatics and in 7% of children regarded as "healthy" at the end of the investigation period Asthma seemed to be predicted better by a high serum IgE value than by eosinophilia (>400/mm³) but the reverse was found when the number of blood eosinophils was high (>700/mm³)

A variation of the serum IgE concentration with the time of the year was found in all groups but least pronounced in the asthma group The lowest levels were found at the beginning of the year and the highest values in the autumn

ACKNOWLEDGEMENTS

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The author wishes to express his thanks to Associate Professor Claes Högman, M.D and Assistant Professor S Gunnar O Johansson, M D. for the facilities of their department placed at my disposal. I also thank Professor Stig Sjölin, M.D and Assistant Professor Torsten Berg, M D for their practical support of this investigation, Göran Engholm M.A for his help with the statistical problems and Mrs Helena Norlm and Mrs Stina Söderlund for skilful technical assistance.

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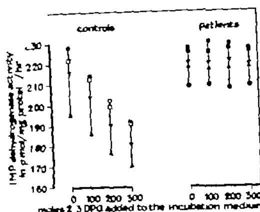


Fig 1 Influence of 2,3 DPG on IMP dehydrogenase activity Each symbol represents one individual.

throcytes were frozen at −20°C, thawed, and the resulting hemolysate was dialysed against 0.066 M Tris-HCl *buffer* pH 8.0.

IMP dehydrogenase was partially purified from the hemolysate as described by Pehlke et al. (6). Contrary to this method, a 0.03 M potassiumchloride solution in 0.066 m Tris HCl buffer pH 8.0 was used instead of 0.05 M sodiumphosphate buffer pH 7.4 and after hemoglobin was removed from the *column*, the IMP *dehydrogenase* was obtained by adding 0.3 M potassiumchloride solution in 0.066 M Tris-HCl buffer pH 8.0. The protein content was determined according to the method of Lowry et al. (4)

IMP dehydrogenase activity was measured according to the method of Magasanik (5). Each incubation *mixture* contained ³H-8-IMP 18.2 nmoles, NAD 0.2 μmoles, glutathione 0.1 μmoles, KCl 10 μmoles, Tris-HCl (pH 8.0) buffer 10 μmoles and a specified amount of enzyme preparation in a total volume of 220 μl. Incubation was performed for 3 hours at 37°C and the reaction was stopped by addition of 20 μl TCA 5%. Protein was removed by centrifugation at 1 200 *g for 20 min. 20 μl of the supernatant* was spotted on Whatman DE-81 paper with carrier XMP GMP *and* IMP and *developed in an* ascending system *in* 0.2 M ammoniumformate buffer pH 5.0. After development, the spots were detected with *an ultra*violet light source, cut out, and after treatment of the paper pieces with Nuclear Chicago Solubilizer the radioactivity was measured in Nuclear Chicago Liquid Scintillation Counter

RESULTS

Fig 1 shows the results of the IMP dehydrogenase assay of the partially purified protein fraction from normal erythrocytes (4 individuals) and HGPRT-ase deficient erythrocytes (5 patients) both with and without addition of 2,3 DPG It appears that the enzyme of HGPRT-ase deficient erythrocytes and of normal erythrocytes shows almost the same activity without addition of 2,3 DPG After addition of 2,3 DPG however the activity of the enzyme obtained from normal erythrocytes shows a decrease proportionally to the amount of 2,3 DPG whereas the activity of the enzyme obtained from HGPRT-ase deficient erythrocytes shows almost no decrease under the same circumstances.

In a separate experiment we investigated the influence of very high concentrations 2,3 DPG (5 000 nmoles added to the incubation medium) In one of the controls we found a decrease of activity of 70% whereas in 2 of the patients no inhibition was found.

DISCUSSION

These findings seem to be in contradiction with the conclusion of Pehlke et al. (6) that the increased IMP dehydrogenase activity in HGPRT-ase deficient erythrocytes is due to the absence of an unidentified dialyzable inhibitor which is present in normal erythrocytes.

It is suggested that in HGPRT-ase deficient erythrocytes the IMP dehydrogenase is altered with regards to its sensitivity for inhibition by 2,3 DPG The difference in activity is probably not due to the absence of an inhibitor in HGPRT-ase deficient erythrocytes but will be caused by an altered IMP dehydrogenase which is not inhibited by 2,3 DPG

In the afore-mentioned experiments the concentration of 2,3 DPG is about 5 to 15 times that of the substrate IMP except for the separate experiment where 5 000 nmoles of 2,3 DPG were added, resulting in a 2,3 DPG concentration of 250 times that of IMP This 2,3 DPG/IMP ratio approximates the ratio in circulating erythrocytes where IMP is not detectable (below 1 μmoles per 1 of free cell water), (3) and the concentration of 2,3 DPG is 5 000 μmoles per l of free cell-water Thus in normal erythrocytes IMP dehydrogenase

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THE IMP DEHYDROGENASE CATALYSED REACTION IN ERYTHROCYTES OF NORMAL INDIVIDUALS AND PATIENTS WITH HYPOXANTHINE GUANINE PHOSPHORIBOSYLTRANSFERASE DEFICIENCY

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Erythrocytes of patients with the Lesch Nyhan syndrome deficiency of hypoxanthine guanine phosphoribosyltransferase (HGPRT-ase deficiency) show a decreased concentration of adenosinetriphosphate (ATP) adenosinediphosphate (ADP) and adenosinemonophosphate (AMP) and a normal concentration of guanosinetriphosphate (GTP) In our experiments no other purine nucleotides have been detected in erythrocytes (3) It was concluded that there was a disturbed balance between the monophosphoribonucleotides AMP inosinemonophosphate (IMP) and guanosinemonophosphate (GMP) as a result of a shift of adenine nucleotides via IMP xanthosinemonophosphate (XMP) towards the guanine nucleotides. This shift disappeared after treatment of the patients with adenine (3)

One of the reactions involved in the conversion of AMP into GMP namely the irreversible oxydation of IMP to XMP is catalysed by inosinic acid dehydrogenase (IMP dehydrogenase) Pehlke et al (6) assayed the activity of this enzyme for the first time in human erythrocytes. They found a significant increased enzymic activity in erythrocytes of patients with HGPRT-ase deficiency as compared with normal erythrocytes. They concluded that this increase is due to the absence of an unidentified, dialysable inhibitor in erythrocytes of patients which is present in normal erythrocytes

Because 2,3 diphosphoglyceric acid (2,3 DPG) is a well known inhibitor of the enzyme adenylic acid deaminase (AMP deaminase) (1), which converts AMP into IMP our attention was focused on the eventual influence of 2,3 DPG on IMP dehydrogenase In erythrocytes 2,3 DPG was found in very high concentrations (about 5 000 μmol/l of free cell water).

We investigated the influence of 2,3 DPG on partially purified IMP dehydrogenase from normal and HGPRT-ase deficient erythrocytes. The clinical features of the 5 patients with HGPRT ase deficiency are reported elsewhere (2,3)

MATERIALS AND METHODS

^{3}H-8-IMP (5.5 Ci/mmol) was obtained from the Radiochemical Centre Amersham, and 2,3 DPG from Boehringer Mannheim GmbH

For the partial enzyme purification, blood from normal donors and patients with the Lesch-Nyhan syndrome was drawn into heparinized tubes (10 units ml) and centrifuged at 1 200 g for 15 min in the cold. Plasma and buffycoat were removed by respiration, the red cells were resuspended in at least 5 vol of cold 0.9% sodiumchloride solution, recentrifuged at approximately 1 000 g for 10 min, and residual buffy coat was removed again. The washed packed ery

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EFFECT OF OROTIC ACID UPON SERUM BILIRUBIN IN NEWBORN INFANTS WITH ERYTHROCYTE G-6-PD DEFICIENCY

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From the University Children s Hospital Sassari Italy

Erythrocyte G-6-PD deficiency B(−) type (13) occurs frequently in Sardinia.

Given this fact, about one-fourth of all ex change transfusions are performed in our University Children s Hospital on mature male newborns. Very little is known about the chain of events which leads to this form of hyperbilirubinemia. Hemolysis, even when present, does not seem to play an important role. Valaes and his associates (11) carried out an extensive survey in this field on the population of three Greek islands where the incidence of G-6-PD deficiency is very similar to that observed in Sardinia. These authors found that the hemoglobin (Hb) and hematocrit (Ht) values in jaundiced newborns with G-6-PD deficiency were slightly below the minimum level observed in unaffected newborns; in many instances, however these findings were normal or above the average level found in the controls. These observations agree with our data (8) as well as with those of Panizon (9) and of Vecchio (12) thus demon strating that in G-6-PD-deficient newborns, severe neonatal jaundice is not to be associated with anaemia. For this reason every attempt to lower the dangerous serum level of bilirubin by enhancing its metabolism, seems to be indicated, since the restoration of erythrocyte loss or the removal of antibodies are not needed, as in blood group incompatibilities.

In the present work the results of our experiments on the effectiveness of orotic acid are reported. This drug, according to the theoretical assumptions advanced by Brodersen (1) and to clinical experiences described in the literature (3 4) is said to be useful in the activation of the bilirubin metabolism of the premature neonate. These results, however have not been confirmed by Gray & Mowat (2)

Two Japanese authors (7) obtained positive results in mature newborns, too. On these grounds, we selected a number of patients for treatment with orotic acid, comparable to those we used for evaluating barbituric acid treatment.

MATERIAL AND METHODS

From November 1971 to January 1972 and from January to March 1973 at the neonatal unit of this University all mature male newborns were investigated immediately after birth for erythrocyte G-6-PD deficiency (5).

Fifty healthy babies with enzyme deficiency but no blood group incompatibilities, respiratory distress, hypoglycemia or cephalhematoma and whose mothers had not been treated during parturition with any drug listed as potentially responsible for hemolysis, were used for this study Twenty-five randomly selected newborn babies were given 100 mg/kg body weight of orotic acid orally (a test preparation of Teknofarma, Torino) in three daily doses, from their 1st to their 5th day of life. The other 25 newborns received no treatment and served as controls.

Venous blood samples for the determination of bilirubin hemoglobin (Hb), hematocrit (Ht) and glucose were collected for the first time within 12 hours of birth and once a day thereafter Bilirubin deter

will be strongly inhibited It is highly probable that the inhibitor proposed by Pehlke et al (6) is identical with 2,3 DPG

Either the IMP dehydrogenase in erythrocytes of HGPRT ase deficient individuals is a *mutant enzyme or there are other factors* causing this remarkable findings has to be subject of further and more comprehensive investigations. An alternative explanation could be that the protein preparation from HGPRT ase deficient erythrocytes is more effective in degrading 2,3 DPG To investigate this possibility mixing experiments are to be done

SUMMARY

This paper deals with a preliminary investigation concerning the possible mechanisms causing an aberrant purine nucleotide pattern in erythrocytes of hypoxanthine guanine phosphoribosyltransferase deficient individuals (Lesch Nyhan Syndrome) The AMP deaminase inhibitor 2,3 DPG has been found to be also a strong inhibitor of IMP dehydrogenase obtained from normal erythrocytes. IMP dehydrogenase from HGPRT-ase deficient erythrocytes, however showed a complete insensitivity for this inhibition

ACKNOWLEDGEMENT

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Venous blood samples for the determination of bilirubin, hemoglobin (Hb), hematocrit (Ht) and glucose were collected for the first time within 12 hours of birth and once a day thereafter Bilirubin deter

Table 1 *Newborns with G-6 PD deficiency*
Treatment with orotic acid from the 1st day of life (100 mg/kg/day)

No	Birth weight (g)	Total bilirubin serum level (mg/100 ml) during first 5 days of life 1	2	3	4	5
1	3 200	1 6	2.8	4 0	5 0	2.6
2	3 000	1 6	2.8	1 6	1 6	1 6
3	3 400	0 8	0 8	0.8	0 8	1.2
4	3 450	3.2	2.8	2.4	1 6	0.8
5	4 600	0 4	1.2	1 6	1 6	0.8
6	3 250	5.2	7.2	14.2	18.2*	
7	3 250	2.0	1 6	2.8	1 6	1 6
8	3 000	5.2	6 0	6 4	4.8	5 6
9	3 100	8 0	9 4	8 0	8.8	6.4
10	3 000	4.8	4 0	9 4	7 6	9 4
11	2 900	2.8	4.0	4.8	7.2	13 0
12	3 900	1.2	1 6	1 6	2.4	0 8
13	3 550	2.4	2.4	8.0	6.4	20 6*
14	3 350	1 6	3 4	3 8	4.2	3 0
15	3 100	2.0	5 6	10 4	16 4	18.2*
16	3 100	1 2	1 6	1 8	2.8	2.8
17	3 250	1 6	1 6	2.4	3.2	2.8
18	3 200	3.2	6.4	8 0	11 4	10 2
19	3 000	5.2	8 4	14 2	18 8	
20	4 800	2.4	7 6	7.2	7.2	4 0
21	3 050	4 0	6 4	4 4	4.8	2.8
22	3 250	3.2	6.0	9 0	12.0	8 0
23	2 950	3 8	10 6	18.2	*	
24	4 100	3 8	11 4	10 2	12.6	10.2
25	2 800	4 0	9 4	11 0	17 4	21 0
Controls						
1	3 300	1 6	2.4	3.2	2.4	2.0
2	3 200	8 8	12.6	16 0	16.8	12.2
3	3 750	6.0	4 4	6.8	5.2	4 0
4	3 850	5 2	5 2	6.0	5.2	4.2
5	4 150	7 6	8 4	9 8	11 4	9 8
6	3 600	5 6	9 0	9 4	8.0	6.0
7	4 100	4.0	6 8	4 0	2.8	2.4
8	3 050	3.2	6.8	25 0		
9	3 000	11 4	6 4	9 6	8 8	7 2
10	3 800	4 2	8 0	15 4	15 0	18 8
11	3 050	2.4	1 6	1.2	1.2	1 2
12	2 800	5 6	8 8	13 8	18.2	
13	3 250	2.8	3 6	6.0	7 6	7 6
14	3 700	0 8	0 8	2.4	2.4	1 6
15	3 550	0 8	11 0	9.8	14.2	15 8
16	3 550	6 8	7 6	10.2	9 6	9 6
17	3 100	0.8	0 8	2.0	0.8	0 8
18	4 000	1 6	6 4	4.0	3 6	4 0
19	3 300	4.0	4 8	11 4	18 8	
20	3 300	4 0	4 0	6.4	4 4	4 4
21	3 200	0.8	0 8	1 6	1 6	0 8
22	3 800	4.2	7 6	10 6	12.6	18 8
23	3 250	4 0	7.2	13 8	15 6	19 6
24	4 250	2.4	3 2	2.8	2.4	2.0
25	3 400	1 8	4 0	2.4	3.2	2.4

* Exchange transfusion.

Table 2 *Geometric mean (shown as log) of the total bilirubin serum levels (mg/100 ml) and the standard deviation of the log of same*

	Day of life 1	2	3	4	5
Treated					
Mean	0.396	0.594	0 696	0.721	0 613
S.D	0.294	0.328	0.365	0.395	0 463
Untreated					
Mean	0 493	0.654	0.791	0.757	0 669
S D	0.339	0.349	0.351	0.402	0 493
t	1.059	0 626	0.938	0 271	0.341

minations were made (in duplicate) by Malloy & Evelyn s method (6). Ht was measured (in duplicate) using Ljungberg Cellocrite microhematocrit tubes. Hb was determined (in duplicate) by Drabkin's method Glucose blood level was controlled using the glucose-oxidase method.

Since the bilirubin levels do not show a normal distribution the daily mean value has been calculated as the geometric mean of the concentrations and the standard deviation estimated from the logarithms of the concentrations given

RESULTS AND DISCUSSION

The bilirubin blood levels in the treated as well as in the untreated newborn babies are shown in Table 1 No significant difference between the two groups resu ed from a statistical evaluation of the geometric mean bilirubin values (Student s *t* test) (see Table 2).

Six exchange transfusions were performed in the first group and six in the second. I is our practice to carry out an exchange transfusion in mature full-term newborns whe the bilirubin blood level independent of an underlying factor reaches 17–18 mg/100 m serum It is evident that orotic acid was no effective in preventing severe hyperbilirubinemia.

It is noteworthy that also in the G-6-PD deficient newborns who underwent exchange transfusion after severe hyperbilirubinemia had occurred the Ht values remained more or less within the normal range (Table 3)

The underlying pathogenetical factors of severe jaundice in G-6-PD-deficient newborns

Table 3 *Haematocrit values in the exchange-transfused newborn babies*

	Day of life				
N	1	2	3	4	5
Group of treated newborns					
6	55	55	54	54	
13	56	56	55	54	54
15	61	59	58	57	57*
19	58	56	55	55*	
23	54	52	52*		
25	62	59	58	57	56*
Group of controls					
8	56	52	52*		
10	52	52	50	50	50*
12	55	54	53	53*	
19	60	59	58	56	
22	59	57	56	54	53
23	57	57	56	56	54*

* Exchange transfusion.

in Sardinia, where the enzymatic defect is an overall B(−) genetic type seem paradoxically to be of extracorpuscular origin, too We have already noted in a previous paper (8) that this phenomenon has not been accounted for One might surmise that G-6-PD deficiency of the B(−) type, which is not confined to the erythrocytes but has also been detected in the liver (10), might play an additional role in worsening the ability of the physiologically impaired neonatal liver to metabolize bilirubin.

An alternative hypothesis is that increased erythrocyte destruction occurs during the first days of life, or immediately prior to birth. Such a possibility however cannot be demonstrated unequivocally by Hb or/and Ht determinations

The question therefore arises: Is it possible that, in the mature newborn with normal glucose blood level, in the absence of acidosis, a slight reduction in the erythrocyte mass might be sufficient to cause severe jaundice?

In our experience, whatever the pathogenesis of this form of jaundice may be, barbiturates are certainly very effective in enhancing the bilirubin clearing process of the liver cells and in preventing hyperbilirubinemia in mature G-6-PD-deficient newborns. In a previous paper (8) we reported the results of prophylactic treatment with phenobarbitone in a group of newborn infants with G-6-PD deficiency In the test group no exchange transfusions were needed, in the control group, however several babies required one.

SUMMARY

Fifty mature male newborns with erythrocyte G-6-PD deficiency were used for a study concerning the effectiveness of orotic acid in preventing severe hyperbilirubinemia. Twenty five randomly selected neonates were given orotic acid (100 mg/kg/day) orally in two daily doses from their 1st to their 5th day of life. Twenty-five newborns were not treated and served as controls. Six exchange transfusions were performed in the test group and six in the controls.

According to these results orotic acid does not seem to be effective in preventing severe hyperbilirubinemia, which frequently occurs in newborn babies with erythrocyte G-6-PD deficiency

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Key words. Orotic acid erythrocyte G-6-PD deficiency bilirubin

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CASE REPORT

SEA BLUE HISTIOCYTE SYNDROME

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The sea blue histiocyte syndrome, first described in 1950 has received increasing attention during the last few years (13 20 21). So far 39 cases have been reported, most in the last two years (1 3 8 14) however only one in pediatric literature (2). Among those, 23 patients fulfilled the criteria of primary syndrome of the sea-blue histiocyte (16). In reporting three new cases of primary syndrome which appeared in different clinical presentations we aim to bring this syndrome to the attention of more pediatricians.

PATIENT REPORTS

Patient 1 O. G. (HCH 67 69972), a 13-year-old female was seen at Hacettepe Children's Hospital because of growth retardation, epistaxis, and a protuberant abdomen. The enlarged spleen was first palpated at two years of age. Since then, abdominal protuberance has become more prominent and she has had intermittent, brief episodes of epistaxis. Her growth retardation has become more marked in recent years.

Physical examination revealed a poorly developed female whose weight (29 kg) and height (127 cm) were both below the third percentile. Secondary sex characteristics were absent. The head measured 50.5 cm in circumference and the abdomen 70 cm. The skin was dry, the hair sparse. There was seborrhea of the scalp and nummular eczema on the right hand. The liver was palpable 5 cm below the right costal margin and the spleen was down to the iliac crest. The rest of the findings, including neurologic and eye ground, were noncontributory.

Pertinent laboratory findings are shown in Table 1. Formol-gel, VDRL, and L.E. cell preparations were negative and P.B.I. (4.1 μg/100 ml) was normal. Chest and long bone surveys revealed no abnormalities, however, the bone age corresponded to that of a 9-year-old. The bone marrow, spleen, and liver needle biopsies stained with Wright stain revealed many sea blue histiocytes. Liver biopsy also revealed cirrhosis.

The parents were not blood relatives, and her three siblings were apparently normal.

Patient 2. O. K. (HCH 204289), a 3-year-old male was brought to the hospital because of abdominal swelling and frequent epistaxis of one year's duration. Prenatal, natal, and immediate postnatal histories were not contributory. The parents were first cousins.

Physical examination revealed an alert, pale, underdeveloped boy (weight 8.7 kg, height 80 cm, both below the third percentile) whose abdomen was markedly distended. A 3/VI systolic murmur was noted over the precordial area. The liver and spleen were palpable 7 cm and 6 cm below the respective costal margins. No ascites, hemorrhoids, or collateral circulation were found, and auscultation revealed no periumbilical murmur. Other findings including eye grounds and complete neurologic examination were within normal limits.

Laboratory findings are given in the table. The erythrocytes were hypochromic and microcytic. Chest and skull X-rays were negative. No esophageal varices were demonstrated on barium swallow. Bone marrow with Wright's stain revealed sea blue histiocytes and moderate erythroid hyperplasia, maturation of all elements and cellularity were normal.

Neither of the patient's parents nor a sister had hepatosplenomegaly and bone marrow from both parents showed no sea blue histiocytes.

Patient 3. Z. S. (HCH 179309), a 14-year-old male was seen at this hospital because of mental retardation. He had difficulty in learning from the early childhood. His spleen was found to be enlarged at the age of about 3 years when he was examined because of fever.

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[2] Associate in Paediatrics and Fellow in Paediatric

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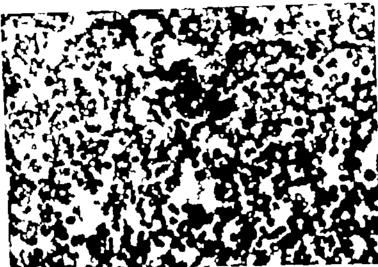


Fig. 1 Different kinds of sea blue histiocytes in the splenic needle biopsy of patient Z. S. In the upper middle: a histiocyte with large homogenous blue staining granules, in the lower middle, a histiocyte with fewer granules; the cells at the periphery somewhat resemble Niemann–Pick cells.

to chance alone (2, 5 12, 22). Sea blue histiocyte syndrome associated with mental retardation (12) and/or neurologic diseases (7) has also been described as in one of the present cases.

The storage histiocytes in the bone marrow and spleen were not all morphologically same, which was indicated previously (13 15 17 22). One cell type was packed with large, homogenous blue-staining granules, others contained fewer granules, and occasional histiocytes contained no granules at all (Fig. 1). There is dispute about this syndrome in the literature (11) and the chemical content of the lipid of these cells is also under discussion (10, 11 13–15 17). Phospholipids, especially sphingomyelin, and glycolipids are increased (2, 15–17 19) and the blue staining character of granules appears consistent with the formation of ceroid by the peroxidation and polymerization of these lipids (10).

Petechiae, due to hypersplenism related thrombocytopenia, have been known in this syndrome. Two of our patients had history of epistaxis. Although detailed coagulation and qualitative platelet studies were not carried out their platelets were adequate on the smear and the screening tests of coagulation did not disclose any abnormality

The growth retardation has, also, been reported in children with the congenital form of the syndrome (4 12), as in our patients, which may be the reflection of a basic metabolic alteration in patients with this syndrome. This assumption and the appearance of different clinical varieties will most likely be explained by the ascertainment of the exact concentration of stored lipids and their excess production.

SUMMARY

Three children with congenital form of sea blue histiocyte syndrome are reported. Two of them had liver involvement and the third one was mentally retarded. In all these cases, marked growth retardation was documented and the two had frequent epistaxis. The diagnosis was made by the demonstration of sea blue histiocytes in the bone marrow These cells were also shown in the liver and spleen needle biopsy specimens, whichever was obtained.

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Table 1 *Pertinent laboratory findings*

	O G	O K	Z S
Hemoglobin (g/100 ml)	11 79	7 98	13 1
Hematocrit (%)	N D[a]	N D	39
Leukocytes (mm³)	16 800	10 800	9 900
Platelets	abundant	abundant	adequate
Quick Prothrombin Time (< 12 sec)[b]	11	17	15
PTT (60–120 sec)	N D	112	116
Albumin/globulin (g/100 ml)	5/2.3	5.5/3.2	5.2/2.8
Serum electrophoresis ( )			N D
Albumin	37	32	
α_1	4	2	
α_2	17	14	
β	15	18.8	
γ	23	32.2	
SGOT (< 40 Karmen units)	82	150	28
SGPT (40 Karmen units)	70	98	18
Thymol turbidity (< 5 u)	N D	16	1
Cholesterol (150–250 mg/100 ml)	208	250	N D
CCF (+ to +)	+	+	N D
Alkaline phosphatase (< 5 Bodansky)	3.2	N D	N D
Total lipids (700–110 mg/100 ml)	800	N D	N D
Calcium (9–11 mg/100 ml)	9 4	N D	9
Phosphorus (4–6 mg/100 ml)	4 9	N D	4 7
N P N (40 mg/100 ml)	24	N D	45

[a] N D not determined
[b] Values in parenthesis indicate our normals.

Physical examination revealed a markedly mentally retarded preadolescent male weighing 33 kg and measuring 133 cm (both below the third percentile). The spleen was palpable 7 cm below the left costal margin and the edge of the liver could be felt at the right costal margin. All the other findings including eye grounds and neurologic examination were normal.

Skull chest, and wrist X-rays disclosed no abnormality Bone marrow and splenic needle biopsies revealed abundant sea blue histiocytes with Wright stain but were otherwise normal Other laboratory results are summarized in the table

The parents were first cousins. The patient's five sisters and one brother appeared normal to the parents. Only the father was available for examination. He did not have hepatosplenomegaly and refused bone marrow examination

Neither of these three patients had abnormal skin pigmentation, enlarged lymph nodes, pingueculae and no history of jaundice convulsion petechiae ecchymoses, hematemesis melena, neonatal umbilical infection or exposure to toxins could be elicited The sea blue histiocytes in the bone marrow and other tissues of these patients were PAS positive and were reactive with scharlach Sudan black and oil red 0. These histo-chemical reactions are consistent with the diagnosis.

COMMENTS

Sea blue histiocyte syndrome is a lipid storage disorder of unknown etiology which can be either congenital (primary) or acquired (secondary) The secondary form is most commonly observed in adults who have haematologic diseases such as sickle-cell anemia, thrombocytopenic purpura, thalassemia, or myeloproliferative disorders (5 6, 8 11 18), and in patients with familial lecithin-cholesterol acyltransferase deficiency (3). In the patients with the acquired type the spleen may be of normal size the serum cholesterol is usually low and the histiocytes are found in the bone marrow but rarely in the spleen and/or liver (5 6 8 11 16)

The congenital forms is observed most frequently in children and young adults, for which autosomal recessive mode of inheritance has been suggested (4 5 7 16 22). Two of our patients genetic data is consistent with this evidence being offspring of consanguineous marriages.

The congenital form of this syndrome is characterized by splenohepatomegaly and histiocytes containing granules which stain bright blue or greenish-blue with Wright and/or Giemsa stain in bone marrow spleen and less frequently in liver and lymph nodes (16). The findings in our patients are consistent with the criteria for the congenital form of this syndrome The absence of hematologic disorders except for probable iron-deficiency anemia in one patient is also in favor of the congenital form

Clinically the patients with congenital sea blue histiocyte syndrome have a relatively benign course though mild hypersplenism may sometimes develop Most of the reported patients showed no functional or architectural abnormality of the liver But in few liver involvement with (13 15 17) or without (2, 5 12, 22) cirrhosis has been reported as was shown in two of our patients. Cirrhosis of the liver in children is not rare in Turkey (9), but the appearance of both of these disorders in the same patient can not be attributed

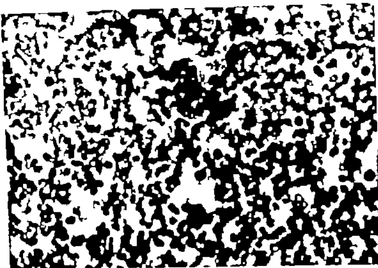


Fig. 1 Different kinds of sea blue histiocytes in the splenic needle biopsy of patient Z. S. In the upper middle a histiocyte with large, homogenous blue staining granules, in the lower middle, a histiocyte with fewer granules; the cells at the periphery somewhat resemble Niemann–Pick cells.

to chance alone (2, 5 12, 22). Sea blue histiocyte syndrome associated with mental retardation (12) and/or neurologic diseases (7) has also been described as in one of the present cases.

The storage histiocytes in the bone marrow and spleen were not all morphologically same, which was indicated previously (13 15 17 22) One cell type was packed with large, homogenous blue-staining granules; others contained fewer granules and occasional histiocytes contained no granules at all (Fig. 1). There is dispute about this syndrome in the literature (11), and the chemical content of the lipid of these cells is also under discussion (10 11 13–15 17). Phospholipids, especially sphingomyelin, and glycolipids are increased (2, 15–17 19) and the blue staining character of granules appears consistent with the formation of ceroid by the peroxidation and polymerization of these lipids (10)

Petechiae due to hypersplenism related thrombocytopenia, have been known in this syndrome. Two of our patients had history of epistaxis. Although detailed coagulation and qualitative platelet studies were not carried out their platelets were adequate on the smear and the screening tests of coagulation did not disclose any abnormality

The growth retardation has, also, been reported in children with the congenital form of the syndrome (4 12) as in our patients, which may be the reflection of a basic metabolic alteration in patients with this syndrome. This assumption and the appearance of different clinical varieties will most likely be explained by the ascertainment of the exact concentration of stored lipids and their excess production.

SUMMARY

Three children with congenital form of sea blue histiocyte syndrome are reported. Two of them had liver involvement and the third one was mentally retarded. In all these cases, marked growth retardation was documented and the two had frequent epistaxis. The diagnosis was made by the demonstration of sea blue histiocytes in the bone marrow These cells were also shown in the liver and spleen needle biopsy specimens, whichever was obtained.

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Table 1 *Pertinent laboratory findings*

	O G	O K.	Z S
Hemoglobin (g/100 ml)	11 79	7 98	13 1
Hematocrit ( )	N D[a]	N D	39
Leukocytes (mm)	16 800	10 800	9 900
Platelets	abun-dant	abun-dant	ade quate
Quick Prothrombin Time (< 12 sec)[b]	11	17	15
PTT (60–120 sec)	N D	112	116
Albumin/globulin (g/100 ml)	5/2.3	5.5/3.2	5.2/2.8
Serum electrophoresis ( )			N D
Albumin	37	32	
α_1	4	2	
α_2	17	14	
β	15	18 8	
γ	23	32.2	
SGOT (< 40 Karmen units)	82	150	28
SGPT (40 Karmen units)	70	98	18
Thymol turbidity (< 5 u)	N D	16	1
Cholesterol (150–250 mg/100 ml)	208	250	N D
CCF (to + +)	+		N D
Alkaline phosphatase (< 5 Bodansky)	3 2	N D	N D
Total lipids (700–110 mg/100 ml)	800	N D	N D
Calcium (9–11 mg/100 ml)	9 4	N D	9
Phosphorus (4–6 mg/100 ml)	4 9	N D	4 7
N P N (< 40 mg/100 ml)	24	N D	45

[a] N D – not determined.
[b] Values in parenthesis indicate our normals.

Physical examination revealed a markedly mentally retarded preadolescent male weighing 33 kg and measuring 133 cm (both below the third percentile) The spleen was palpable 7 cm below the left costal margin and the edge of the liver could be felt at the right costal margin All the other findings including eye grounds and neurologic examination were normal.

Skull, chest, and wrist X-rays disclosed no abnor mality Bone marrow and splenic needle biopsies re vealed abundant sea blue histiocytes with Wright stain, but were otherwise normal. Other laboratory results are summarized in the table.

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CASE REPORT

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From the Department of Paediatrics and Child Care and the Department of Haematology Hadassah University Hospital Jerusalem Israel

Since the first description of non-cirrhotic portal fibrosis in 1962 by Ramalingaswami et al. this syndrome has already become a well established clinico-pathological entity (10) Although its aetiological basis is still not understood, it is characterized by portal hypertension with marked splenomegaly usually of long duration recurrent hemorrhage from esophageal varices and sparse biochemical evidence for parenchymatous liver damage. The histological studies reveal varying degrees of portal fibrosis with small round cell infiltration but without the regenerative nodules alongside destruction of liver structure found in classical liver cirrhosis.

This report of a similar case was encouraged by some anomalies found in the coagulation mechanism of the patient, suggestive of a chronic consumptive coagulopathy which may contribute to the understanding of the pathogenesis of this obscure illness.

CASE REPORT

A 1 -year-old girl, born of healthy unrelated parents in Hebron was referred to us because of marked abdominal distension. She was the fourth of nine children, three of whom had died from unknown causes, while the remaining five siblings were healthy The propositus developed uneventfully until the age of years, when the distention of her abdomen was first noticed by the family Medical advice, however was sought only 9 years later and then for upper respiratory tract infection.

Physical examination revealed a markedly lean girl The only positive finding was a firm, non-tender spleen, extending 15 cm below the costal margin.

Laboratory findings

There was a moderate anemia. The number of white cells as well as of the platelets was moderately decreased (Table 1). The differential count was normal. The peripheral blood smear revealed marked anisocytosis, poikilocytosis and moderate hypochromia. The serum iron concentration was low with a high unsaturated iron binding capacity (8) and whole blood L. casei folate activity was also decreased. The ^{51}Cr labelled red cells (3) were removed from the circulation slightly faster than the normal (T½ = 22 days). The bone marrow smear was cellular All forms were proportionately represented, except for increased number of megakaryocytes and lymphocytes.

The results of the liver function tests performed are summarized in Table 2. There was a mild increase in the amount of bromsulphthalein retained in the plasma 45 minutes after its injection; otherwise no anomalies could be seen.

A slightly elevated serum concentration of IgG was found (1 800 mg/100 ml) associated with normal IgA and low IgM levels. Laboratory tests reflecting kidney function, the intestinal flora, serology (Salmonella, Brucella, Rickettsia and others) were negative. Neither antinuclear factor nor L. E. cells were demonstrable.

Portal venography disclosed a marked dilatation of the main portal and splenic veins with poor visualization of the peripheral vascular tree. Portal pressure was 300 mm H_2O. Histological examination of liver sections obtained on open biopsy revealed well preserved hepatic structure except for a varying degree of fibrosis in the portal spaces infiltrated by small round cells. There were no pseudolobules and no obstructive lesions were seen in the portal veins (Figs. 1 2, 3). No esophageal varices were definable radiologically

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Table 3 *Summary of coagulation studies*

	Patients' values	Normal values
Thrombocytes	54 000–92 000/mm³	200 000–400 000/mm³
Bleeding time	2 min 35 sec	3 min
Tourniquet test	Negative	
Fibrinogen	55–65 mg/100 ml	160–300 mg/100 ml
Clotting time	9 min	<8 min
Prothrombin time (Quick)	1 –35 % (after Vit. K, 28 %)	100 %
Partial thromboplastin time (P T T.)	74–100 sec	37–40 sec
Euglobulin lysis time	Start, 50 min; completed 3 / hr	Start, 24 hr completed 5 hr
Factor II	46 %	75–100 %
Factor VII	45 %	75–100 %
Factor V	42 %	75–100
Factor VIII	360–550 %	75–100 %
Factor X	40 %	75–100 %
¹²⁵I Fibrinogen survival ($T\frac{1}{2}$)	14.5 hr	22 hr

deed, the architecture of the liver was well preserved except for portal fibrosis and the regenerative nodules reflecting classical cirrhosis were absent.

Portal hypertension, however was clearly manifest, although without either intra hepatic or extra-hepatic obstruction being demonstrable. Hepatic functions showed only minimal impairment and there was no bleeding tendency

The presence of splenomegaly suggested the possibility of hypersplenism being a major factor underlying the thrombocytopenia. The low level of fibrinogen, however could be explained neither by hypersplenism nor by so mild a degree of hepatic damage or apparent dysfunction

Another possibility was considered to explain the joint occurrence of thrombocytopenia and hypofibrinogenemia, namely consumption coagulopathy In contrast to the acute variety of this process which is characterized by very low levels of coagulation factors, frequently associated with severe bleeding tendency and shock (6) the chronic form is usually benign without apparent bleeding phenomena, and the diagnosis is made only by the demonstration of the anomalous features in the coagulation system (7)

In the case reported here the thrombocytopenia, decreased plasma concentration of the coagulation factors, low plasma fibrinogen content coupled with a shortened life span of the latter suggested the presence of consumption coagulopathy The moderate elevation of the fibrinogen split products and the lack of response to parenteral Vit. K further supported this contention.

The pathogenesis of non-cirrhotic portal fibrosis is unknown. Among several hypotheses which have been proposed are: (*a*) Umbilical sepsis with thrombophlebitis and repeated embolization to the portal circulation. (*b*) An unknown toxin with a specific action on the portal vein radicles and space of Disse (10).

The coagulopathy described in this case could support either of the above hypotheses as it is known that both sepsis and toxins may induce consumption coagulopathy The findings of Sama et al. (10) who demonstrated occlusive changes in the intrahepatic portal vein radicles of some of their patients with this disease is also compatible with our interpretation of the data. While such lesions were not detected in our patient, this does not wholly exclude the existence of chronic consumption. A persistent breakdown of fibrin clots by secondary fibrinolytic activity has been well documented in such disorder (7)

SUMMARY

A 12 year-old girl suffering from non-cirrhotic portal fibrosis is presented. The clinical picture is characterized by splenomegaly without hematemesis and ascites. Portal hypertension was found without evidence of intra or extra-hepatic obstruction. Liver function test results were mildly disturbed. Open liver biopsy revealed fairly well preserved structure of the liver except for increased fibrosis confined to the portal spaces but without characteristic cirrhotic changes.

Table 1 *Summary of hematological data*

R B C	2 850 000 mm³
Hemoglobin	9 8–10.8 g/100 ml
Hematocrit	33–37 %
W B C.	3 600–6 650/mm³ (differential count normal)
Thrombocytes	54 000–92 000/mm³
Reticulocytes	0 8
E S R	6/21
Serum iron	47 μg/100 ml (normal 80–160 μg/100 ml

Table 2. *Summary of liver function tests*

Total protein	6.7 g/100 ml
Albumin	4 g/100 ml
Globulin	2.7 g/100 ml
Cholesterol	180 mg/100 ml
Bilirubin (total)	0 9–1 4 mg/100 ml
Alk. phosphatase	140–210 I.U (Normal, 100 I.U)
S G O T	90–115 U (Normal, 50 U)
Vit B 12	820 pg/ml
B.S P	10 %
Fibrinogen	55–65 mg/100 ml
Prothrombin time (Quick)	12–35
Blood ammonia	22 μg/100 ml

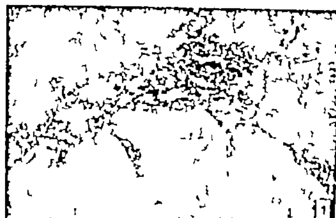
Coagulation studies (Table 3)

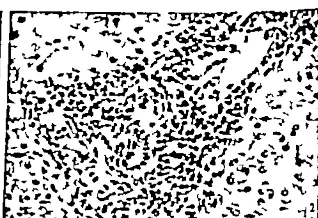
There was a persistent thrombocytopenia and hypofibrinogenemia (9). The one-stage prothrombin (Quick) was prolonged and did not increase after administration of Vit K (menadione sodium bisulph. 20 mg/day I M for 10 days)

The partial thromboplastin time was prolonged (4). There was a moderate to-marked decrease in the activities of factors II (13) V (11) VIII (91) and X (2) The euglobulin lysis time (12) was moderately shortened Iodinated (¹²⁵I) human fibrinogen (Radiochemical Centre Amersham, England 100 μCi) was injected intravenously and the rate of disappearance of the labelled fibrinogen was monitored over a 72-hr period the $T\frac{1}{2}$ was determined by the decay curve plotted on a semilogarithmic paper (5) The fibrinogen survival in this case was reduced by 30 % than the survival in the normal control The amount of fibrinogen degradation products in the patient's serum was moderately elevated 50 μg/ml (6)

DISCUSSION

The child described here conformed to the criteria of the syndrome designated as noncirrhotic portal fibrosis and was characterized by a relatively benign course expressed by slow progress of the disease as well as by splenomegaly without hematemesis, ascites or other features of severe hepatocellular injury In-





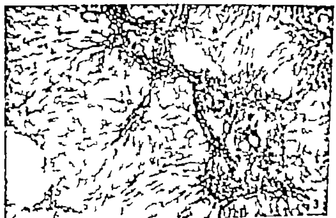


Fig 1 Azan blue stain. Fibrosis of portal spaces with infiltration of small round mononuclear cells.

Fig 2 Hematoxylin-eosin stain. Infiltration of portal spaces with small round mononuclear cells.

Fig 3 Reticulum stain. Fibrosis of portal spaces. No regenerative nodules to be seen. Well preserved hepatic architecture.

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The coagulopathy described in this case could support either of the above hypotheses as it is known that both sepsis and toxins may induce consumption coagulopathy The findings of Sama et al. (10) who demonstrated occlusive changes in the intrahepatic portal vein radicles of some of their patients with this disease is also compatible with our interpretation of the data. While such lesions were not detected in our patient, this does not wholly exclude the existence of chronic consumption A persistent breakdown of fibrin clots by secondary fibrinolytic activity has been well documented in such disorder (7)

SUMMARY

A 12 year-old girl suffering from non-cirrhotic portal fibrosis is presented. The clinical picture is characterized by splenomegaly without hematemesis and ascites. Portal hypertension was found without evidence of intra or extra-hepatic obstruction. Liver function test results were mildly disturbed. Open liver biopsy revealed fairly well preserved structure of the liver except for increased fibrosis confined to the portal spaces but without characteristic cirrhotic changes.

Coagulation studies revealed disturbance of various coagulation factors the most important being thrombocytopenia hypofibrinogenemia and low levels of factors II VII and X It is suggested that the primary underlying cause for these findings is a chronic consumptive coagulopathy and not liver disea e

Appreciation of the coagulopathy accompanying this syndrome may contribute to our understanding of the pathogenesis of this entity

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Key words. Portal fibrosis, coagulopathy splenomeg

CASE REPORT

COLONIC PERFORATION SECONDARY TO THROMBO-EMBOLUS FROM AN UMBILICAL ARTERY CATHETER

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From the Departments of Surgery Pathology II and Paediatrics University Hospital of Umeå Sweden

At Umeå University Hospital a newborn infant was treated for colonic perforation probably following catheterization of an umbilical artery To our knowledge no such case has previously been described.

CASE HISTORY

The patient was a full-term male infant weighing 3 360 g at birth and measuring 52 cm. He was the first child of a 38-year-old woman delivered by caesarean section The infant was considered to be a risk-baby and an indwelling catheter was inserted through the umbilical artery Gas analysis of blood withdrawn through the catheter showed slight acidosis. A 10% glucose solution was administered by way of the catheter for the next 48 hours, after which time the catheter became occluded. Attempts to flush it with saline failed, and it was removed. At the tip there was a blood clot. Later the same day the infant vomited, the abdomen was greatly distended and dilated veins were present on the abdominal wall. X-ray examination of the abdomen revealed free gas and laparotomy was immediately performed.

On the anterior aspect of the caecum there was a 4 mm wide perforation. There was no macroscopically observable inflammation in the surrounding intestinal wall. The wall of the distal ileum was oedematous and its surface was lightly coated with fibrin. Ileo-caecal resection with an end-to-end anastomosis was carried out and the postoperative course was uneventful

Histopathological examination

A total of seven paraffin-embedded tissue specimens were examined. Three of them in serial sections. Sections 6 μ thick were stained with hematoxylin and eosin and with van Gieson's stain.

The submucosa and mucosa of both the caecum and the immediately adjacent part of the ileum were oedematous and hemorrhagic (Fig. 1 *a*). In the area nearest the perforation in the caecum the intestinal wall was necrotic with some evidence of hemorrhage and an accumulation of granulocytes extended to the edge of the perforation (Fig. 1 *b*). However there were only a few distended blood vessels in that area, which explains the absence of a macroscopically visible inflammatory reaction around the perforation. The mucosa and submucosa of the ascending and transverse sections of the colon were normal. The appendix was normal except for slight mucosal bleeding.

DISCUSSION

During the last few years many authors have reported intestinal perforations in newborns in connection with exchange transfusions (1 2, 3) and after infusions into the umbilical vein (6), but there seem to be no reported cases of intestinal perforation following catheterization of the umbilical artery According to Jacobsson & Schlossman (4) it must be assumed that thrombi will form on all indwelling vascular catheters which remain in site for a number of days. Neal et al. (5) have recently shown that thrombo-embolism is a very common complication of catheterization of the umbilical artery In one of their patients there was complete occlusion of the superior mesenteric artery with massive necrosis of the small bowel a few hours after removal of the catheter

Coagulation studies revealed disturbance of various coagulation factors, the most important being thrombocytopenia hypofibrinogenemia and low levels of factors II VII and X. It is suggested that the primary underlying cause for these findings is a chronic consumptive coagulopathy and not liver disease.

Appreciation of the coagulopathy accompanying this syndrome may contribute to our understanding of the pathogenesis of this entity.

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

Meeting in Hamburg, September 8–9 1972

CHRONIC HEPATITIS AND ALLIED DISORDERS

B. Alagille (Paris) *An introductory review of the classification and clinical evolution of chronic hepatitis in children*

At present, chronic hepatitis in children can be divided into three groups, the term chronic meaning a history of hepatitis of more than 1 year

(*a*) Biliary cirrhosis, with evolutive interlobular biliary atresia. its relation to acute hepatitis is generally admitted, although still under discussion

(*b*) Persistent hepatitis (chronic inactive hepatitis)

(c) Chronic active hepatitis (lupoid hepatitis, active juvenile cirrhosis, chronic hepatitis with hyperglobulinaemia, auto-immune hepatitis, chronic viral hepatitis, plasma-cell hepatitis)

The former clinical or biological features described by turns as belonging specifically to one of these three types of chronic hepatitis are no longer considered for diagnosis or prognosis. On the other hand, everyone agrees with the importance of the histological findings, especially in differentiating persistent or non-aggressive hepatitis from chronic active hepatitis, with the histological changes of aggressivity These were defined in 1967 at the Meeting of the European Association for the Study of the Liver in Gothenburg.

In a group of 35 children at first classified as having chronic hepatitis" study of the histological patterns revealed that only 16 actually had chronic hepatitis. 3 persistent non-aggressive hepatitis (1) 6 chronic aggressive hepatitis (2 *a*) and 7 chronic aggressive hepatitis (2 *b*) As previously reported, there was a prevalence of females (12 girls, 4 boys). The incidence increased from 2 to 15 years. A history of acute hepatitis was found in only 4 of the 16 children. Australia antigen was positive in only 3 out of 9 children tested.

In our patients, persistent non-aggressive hepatitis *had a benign course*, whereas chronic active hepatitis with histological aggressivity had an extremely severe course. For this reason massive and prolonged corticosteroid therapy had to be given to the latter group, since the few control studies demonstrated longer survival. But the difficulties of this treatment have to be emphasized frequent clinical and biological checks, frequent hospitalization for histological studies, extreme difficulty about the decision to stop the treatment. However the precise value of immunosuppressors still has to be demonstrated.

W Baumann & M Neidhardt (Mainz) *Chronic active hepatitis in childhood. Pathogenesis immunological and biochemical findings*

The clinical syndrome of chronic active hepatitis (chronic aggressive hepatitis) must fulfil well-defined morphological criteria. Con-

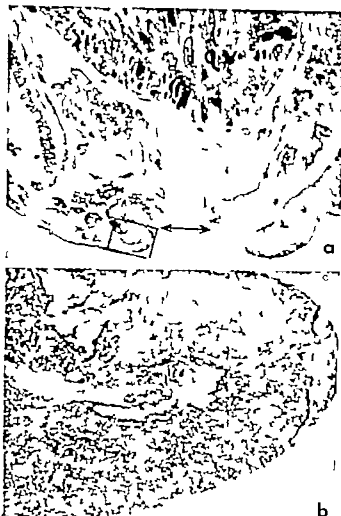


Fig 1 (*a*) Low-power view of the caecum showing the perforation (↔) Outside the necrotic area adjacent to the perforation there is moderat oedema. The ileo-caecal valve (*ICV*) also shows oedema and congestion Van Gieson s stain 16. (*b*) Higher magnification of the area outlined in (*a*) This photomicrograph shows that the small necrotic area of the caecal wall close to the perforation has been densely infiltrated by granulocytes. Van Gieson's stain × 135

In the infant we describe the changes were confined to the ileo-caecal region but in this case as well the perforation seems to have occurred in a diseased colonic wall. The histological picture suggests that there was impairment of the blood supply to the region Although the etiology of the changes is unclear they may have been related to the catheterization of the umbilical artery Embolic material from the tip of the catheter may have followed the main branch of the superior mesenteric artery and lodged at the branch between the anterior and posterior caecal arteries. An embolus in this position could cause considerable circulatory embarrassment in the caecum and the ileo-caecal valve Since the appendical artery usually originates proximal to the caecal artery the circulation to the appendix might remain relatively intact

SUMMARY

A case of colonic perforation in a newborn infant is described. Perforation was probably secondary to an arterial thrombo-embolus originating from a catheter placed in the aorta via an umbilical artery

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Key words. Colonic perforation umbilical artery catheterization

H. W. Rotthauwe (Bonn) *Differential diagnosis of neonatal cholestatic jaundice*

Conjugated hyperbilirubinaemia accompanied by acholic stools in newborn and young infants usually establishes the diagnosis "cholestatic jaundice". Cholestasis is caused by impairment of the bile-secreting apparatus of the hepatocytes. This impairment may be due to a primary metabolic defect in this apparatus. It may also be brought about by mechanical obstruction of intrahepatic and extrahepatic biliary passages. Cholestatic diseases in newborn and young infants without a mechanical obstacle may be due to hereditary metabolic disorders such as galactosaemia and fructose intolerance, or to septicaemia, to the inspissated bile syndrome in severe haemolytic disease of the newborn, or to the heterogeneous group of neonatal hepatitis. The most important types of mechanical jaundice are congenital atresia and hypoplasia of extrahepatic and intrahepatic bile ducts. To ensure appropriate treatment, the objective of diagnostic endeavour should be a rapid and reliable distinction between mechanically and non-mechanically caused cholestasis. The differential diagnosis of neonatal hepatitis of obscure aetiology and congenital biliary atresia may be particularly difficult. For diagnosis, case history details, clinical symptoms and activity of serum enzymes are not of major value. Serum protein reactions are not significantly abnormal. A differentiation between neonatal hepatitis and bile duct atresia has to be achieved by employing the duodenal BSP test and the BRI 131 excretion test, by determination of LP X and alpha-1-antitrypsin in serum, and by percutaneous liver biopsy. Exploratory laparotomy, "open" liver biopsy, operative cholangiography and even dissection of the porta hepatis are indicated, however, if congenital biliary atresia is suspected.

J. F. T. Glasgow (Belfast), D. W. Cox, A. Herez & A. Sass-Kortsak (Toronto): *Alpha-1 antitrypsin deficiency in children with chronic liver disease*

We studied 9 children with alpha 1-antitrypsin (AT) deficiency and chronic liver disease from 8 kindreds. Two apparently distinct forms of clinical presentation were found, 7 patients whose onset was in early infancy with cholestatic jaundice and little evidence of a true hepatitis and 2 slightly older children whose infancy had been uneventful had gastro-intestinal bleeding complicating an already established cirrhosis. Another specific aetiology for the liver disease had been excluded. Liver biopsies during early infancy showed the presence of intra-lobular bile plugs and swollen hepatocytes which contained many diastase resistant PAS-positive inclusions (0.5–5.0 μm). Periportal hepatocytes showed pseudo-acinar formation. There was no bile duct proliferation and little multinucleate giant cell formation.

The serum proteinase inhibitory capacities and AT concentrations of 8 patients (1 had died) were far below the range of normal while those of the parents and many of their sibs lay within an intermediate range. Serum samples have been tested from 40 individuals using acid starch gel electrophoresis and antigen/antibody crossed electrophoresis (1). Six proteinase inhibitor (Pi) types have been found incorporating each of the possible variations of the 3 commonest alleles designated—M, S, and Z. Seven children with liver disease were PiZZ but one other was PiSZ. However, five healthy first degree relatives of these individuals were also ZZ or SZ—hence the importance of environmental or other genetic factors in the pathogenesis of the liver disease.

The course of the illness was variable: 4 children developed portal hypertension and in 2 this was accompanied by hepatocellular failure and eventual death. In the remaining 7 the outlook also varied.

It is suggested that genetic variants of AT other than PiZZ may be associated with liver disease manifesting in several different ways.

ventional microscopy may be supplemented with immunofluorescence techniques. Chronic active hepatitis is now generally considered an auto-immune liver disease Immunological research during the past 15 years has resulted in numerous clinical and experimental findings. In spite of these (sometimes conflicting) data the various mechanisms by which humoral antibodies or cellular factors cause tissue lesions have not yet been definitely elucidated particularly since neither the causative agent nor the primary inducing factor is as yet known Once established the disease often seems to maintain itself although spontaneous remission sometimes occurs. Humoral and cellular immune reactions have considerable pathogenetic and diagnostic value The former (antinuclear factors, anti mitochondrial antibodies, antigamma globulin factors and antibodies against smooth muscle) however are neither organ specific nor disease-specific. On the other hand organ specific auto-antibodies reacting with liver protein are of much greater significance

If one applies all the necessary criteria the syndrome is relatively rare in children. By paying particular attention to this point, we have been able to diagnose 4 cases in the past 2 years. In deciding indications for specific treatment we took particular account of positive humoral and cellular immune phenomena Three patients were treated with a combination of azathioprine and small doses of hydrocortisone Control biopsies after 1 year of immunosuppressive therapy showed nearly normal findings in 2 cases the third which had been histologically far advanced, showed no certain improvement. Cellular immune reactions had become negative in all 3 patients after 1 year while humoral antibodies continued to be present in 1 case Two patients in an advanced stage of the disease are siblings their father also suffers from chronic active hepatitis. The hepatitis-associated antigen was found to be present, and to persist, in all three members of this family This rare observation of familiar chronic active hepatitis may be another illustration of "non parenteral transmission of the hepatitis-associated antigen, as reported in the literature

D Feist (Heidelberg) *Clinical course diagnosis and therapy of chronic aggressive hepatitis in childhood*

In the last 3 years 20 cases of chronic aggressive hepatitis according to the criteria of the European Society for the Study of the Liver were diagnosed by liver biopsy at the Children s Hospital of Heidelberg University Of these 11 were HAA positive and 5 of the HAA negative cases showed symptoms of autoimmunity There was neither hyperproteinaemia nor hypergammaglobulinaemia in about 22% of the HAA-positive cases. Five patients were diagnosed already in infancy The mothers of 3 infants were also HAA-positive but were not themselves suffering from hepatitis. In the actively autoimmune cases, gammablobulin levels ranged between 17 and 58% The immunoglobulines IgG, IgA and IgM were not equally affected by this increase in gammaglobulin and an antinuclear factor was detected in only 2 cases. The first ANF positive patient had ulcerative colitis and Hashimoto-Thyroiditis, the second had a combination of active chronic hepatitis with adrenocortical insufficiency hypoparathyroidism dwarfism and alopecia totalis (= Craig–Schiff–Boone-Syndrome *Amer J Dis Child 89* 669 1955) Therapy with glucocorticoids affected the actively autoimmune cases more rapidly but most of the HAA-positive ones also improved Up to now it has not been possible to discontinue treatment. A 6-year-old HAA positive boy treated with prednisone developed cirrhosis of the left liver lobe while inflammatory activity decreased in the right lobe Additional treatment with D-penicillamine for 5 months resulted in a decrease of the transaminases and of the enlargement of the liver and spleen for the first time.

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studies with purified trypsinogen and enterokinase isolated from the rat or the human intestine were undertaken and the following findings were obtained.

1 Bile acids at a physiological concentration of 2.5 mM increase the initial velocity of the enterokinasecatalyzed trypsinogen activation with a factor of 5 8.

2 In the presence of 2.5 mM bile acids (glycodeoxycholate or taurocholate) the apparent K_m of the enterokinasecatalyzed trypsinogen activation is decreased with a factor of 6.2–6.5 (increased affinity of enzyme towards its substrate).

3. In the presence of the same concentration of bile acids the total amount of trypsin formed from a given amount of trypsinogen is increased with a factor of 3–3.5

These findings strongly support the proposition that the low trypsin activities found in these patients are the result of insufficient trypsinogen activation in the absence of bile and that bile acids are necessary for rapid and complete activation of trypsinogen by intestinal enterokinase.

W J Brzosko & T K Zalewski (Warsava): *Hepatitis B antigen in chronic aggressive hepatitis*

We have used immunofluorescence to examine liver biopsy (85 cases) and autopsy specimens (5 cases) from chronic cases of aggressive hepatitis. *Hepatitis B antigen, HBag,* was present in the hepatocytes in 70% of cases analyzed. Deposits of a mixture of immunoglobulins G and M and occasionally beta 1 C globulin were found in the cytoplasm of HBag-containing hepatocytes, on their plasma membranes, on or in the nuclei, in the cytoplasm of Kupffer cells and, rarely in the sinusoids. The accompanying tissue changes were hepatocellular degeneration and necrosis. The deposits of HBag, identified in extrahepatic sites, including germinal centres of lymph nodes and spleen, kidney glomeruli and blood vessel walls, were as a rule accompanied by deposits of IgG IgM, beta 1C globulin and fibrin. All these deposits showed a strong affinity for guinea-pig complement in the immunohistochemical reaction of complement fixation. Germinal centre activation chronic membraneous glomerulonephritis, panarteritis and simple arteriolar hyalinosis were found at the sites of these deposits.

The presence of lesions in the liver indicates that a humoral immune mechanism plays a substantial role in eliminating hepatocytes infected with hepatitis B virus. The occurrence of extrahepatic lesions associated with deposits of HBag immune complexes is indicative of their inflammatory properties. In the light of the data presented it can be postulated that autoimmunity as a pathogenetic phenomenon in chronic aggressive hepatitis is secondary to virus infection, and its role, if any is also secondary

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IMMUNOLOGIC REACTIONS IN DISEASES OF THE GASTROINTESTINAL TRACT

Joint Meeting with European Society for Paediatric Haematology and Immunology (ESPHI)

Following abstracts are published in Zeitschrift für Kinderheilkunde 113 237 1972.

A. P Douglas (Newcastle) Immunological reactions in coeliac disease

Anne Ferguson & D M. V Parrott (Glasgow) Populations of small intestinal lymphocytes,

G Della Cella, M. Governa & P Durand (Genoa): Antireticulin antibodies in childhood in coeliac disease

J J Baudon, J F Mougenot & N Desquilbet (Paris): The value of serum antibodies and lymphoblast transformation studies as diagnostic tools in gluten sensitive coeliac disease and in cow s milk intolerance,

Reference

1 Fagerhol, M K & Laurell C. B *Clin Chim Acta 16* 199 1967

Ø Aagenæs, K Elgjo E. Munthe & M Fagerhol (Oslo) *Alpha-1-antitrypsin and chronic liver disease in childhood*

Since the report of Sharp in 1969 we have screened our children with liver disease for their alpha 1 antitrypsin (AT) contents in serum Five children with neonatal cholestasis progressing to liver cirrhosis were found to have low AT and Pi type ZZ. Liver biopsies from these children and from four other individuals with Pi type ZZ, and two parents with Pi type MZ were examined with light microscopy electromicroscopy and with fluorescence microscopy after treating the biopsies with fluorescein isothiocyanate labelled Anti-alpha 1 antitrypsin

In all ZZ livers, independent of the presence of liver disease or not strong fluorescence was found intracellularly (present also in a dilution of 1 512) In the adult MZ livers a striking fluorescence was also found (present in a dilution of 1 256 or 1 128) Evidence is presented that the AT deficiency in this syndrome is of the same genetic type as other ZZ s with pulmonary emphysema. All parents were of the genotype MZ.

It is concluded that the pathogenesis of the AT deficiency is a mutation at the locus for the AT's structural genes resulting in an abnormal protein which is released from the liver cells at a markedly reduced rate

E. Eggermont J P Frijns, B van Damme & H Eyssen (Leuven) *The presence of coprostanic acid in the bile of a child with a Zellweger-like syndrome*

This condition was encountered in a girl the first and only child of healthy unrelated parents. At 3 months of age the patient was admitted to hospital because of diarrhoea and jaundice. Her weight was 3 820 g (<P3) and lenght 58 cm (P25). She had a number of congenital anomalies club feet, frontal bossing broad metopic suture, third fontanelle, bilateral epicanthic folds and simian creases The karyotype was normal 46,XX. Histological examination of a liver biopsy specimen suggested that the jaundice was due to cholestasis. Ductular structures were rather sparse. The icterus disappeared at the age of five months. The child failed to thrive was hypotonic and mentally retarded. She died at the age of 8 1/2 months. At that time, her weight was 4 050 g (<P3) and length 65 cm (P10-P3)

The bile acid spectrum of the duodenal fluid was 18% chenodeoxycholic acid, 37% cholic acid and 45 % of an unusual compound not found in control subjects. Using thin-layer chromatography gas liquid chromatography and mass spectrometry the unknown bile acid was identified as trihydroxycoprostanic acid or 3 alpha, 7 alpha 12 alpha-trihydroxy 5 beta-cholestan 26-oic acid.

Obviously the excretion of trihydroxycoprostanic acid was due to reduced capacity of the hepatocytes to split off the 3 terminal carbon atoms of the side chain of trihydroxycoprostanic acid. An alternative hypothesis would be a primary alternation of the cell membrane resulting in premature leakage of trihydroxycoprostanic acid out of the cytoplasm

B Hadorn V Troesch H Götze & S. W Bender (Berne) *Disturbance of trypsinogen activation in children with intrahepatic biliary atresia*

In 4 infants with intrahepatic biliary atresia abnormally low trypsin activities were found in the duodenal contents after stimulation of the pancreas with pancreozymin and secretin. This was not due to generalized pancreatic insufficiency since the other pancreatic enzymes were normal

It was suspected that the low trypsin values were due to insufficient activation of trypsinogen in the absence of bile In vitro

disease or other forms of enteropathy such as postgastroenteritis malabsorption. Dipeptidase activity however varied from nil to normal in apparently similarly affected mucosae.

A similar pattern of unequal loss of function was seen in rats infested with Nippostrongylus braziliensis.

In the jejunal mucosa of children with untreated coeliac disease and other enteropathies, the disaccharidase levels and the uptake *in vitro* of glucose and leucine were below the normal range in all instances. Dipeptidase activities and the uptake of glycine were often within the normal range, however. It seems therefore that the alteration of mucosal function cannot solely be due to loss of enterocytes.

FREE PAPERS

P. A. di Sant Agnese (Bethesda): *Familial inherited pancreatitis: a childhood disease*

Hereditary pancreatitis (HP) is a recently described autosomal dominant disorder of unknown etiology. Symptoms usually begin in childhood, but generally are diagnosed only when pancreatic insufficiency and calcifications appear in the young adult age group. Clinical manifestations include recurrent acute attacks of excruciatingly severe abnominal pain with intercurrent symptom-free periods. In addition to the positive family history there are definite differences in sex incidence, age of onset, occurrence of aetiologic factors (e.g. alcoholism, gallstones), and pancreatic pathology between HP and chronic relapsing pancreatitis of adults. In the course of the disease various complications (e.g. glucose intolerance, pseudocysts, etc.) may occur in HP but less commonly than in classical adult pancreatitis.

Three new kindreds with 30 definite and 51 suspected cases of HP representing more than one-third of all previously reported cases, are presented with detailed investigations of pancreatic and parathyroid function, serum lipids, pathologic specimens, and urinary amino acids. These studies clearly separate HP from diseases otherwise associated with pancreatitis. With these three and the 18 previously reported kindreds, it is evident that HP is not a rare disease and is the most common cause of recurrent pancreatitis in childhood. It should be included in the differential diagnosis of recurrent abnominal pain and pancreatic calcifications in the paediatric age group.

D. N. Challacombe, Judith M. Ricardson, Charlotte M. Anderson & B. Rowe (Birmingham, London): *The contaminated small bowel syndrome in infancy*

This paper describes a qualitative and quantitative study of the aerobic and anaerobic microflora of the upper gastrointestinal tract in three groups of infants.

1. 12 control infants in hospital with disorders unrelated to the gastrointestinal tract.

2. 7 infants with chronic diarrhoea, including cases with chronic non-specific diarrhoea, secondary monosaccharide intolerance and secondary lactose intolerance.

3. 7 infants with prolonged diarrhoea following partial resection of the small or large intestine.

A fine polythene tube weighted with a gold head was used for intubation. The bacterial content of gastric and duodenal juice was cultured. Prolonged duodenal intubation resulted in an absolute increase in total coliform count and emphasised the need to define sampling techniques in this kind of study.

The major bacteriological differences between the three groups were demonstrated in the duodenal cultures. The duodenal juice in control infants was sterile or grew only a sparse microflora free of coliforms. The chronic diarrhoea and post-surgical groups had bacteriological contamination of the duodenum. The microflora in these groups was characterised by increased numbers and types of aerobic and anaerob organisms with E. coli being frequently isolated. The E. coli were

E. Savilahti P Kuitunen & J K Visakorpi (Helsinki) Immunohistochemical study of jejunal biopsies of children with coeliac disease

F Carswell R. W Logan & Anne Ferguson (Glasgow) Reduced concentrations of plasma $beta_{1C}$–$beta_{1A}$ globulins in patients with coeliac disease

R Lagercrantz P Perlmann & S Hammarström (Stockholm) Immunological aspects on ulcerative colitis and Crohn s disease

G de Ritis & J Jos (Paris) *Organ culture of intestinal mucosa in coeliac disease and in cow s milk intolerance Effects of protein fractions*

Intestinal biopsies, obtained from 12 patients (7 children with coeliac disease and 5 with cow s milk intolerance) and 6 normal controls were maintained in culture by a modification of the method described by Browning & Trier (2) biopsies were placed in the organ culture system within 1–4 min after excision and NCTC 109 was added to the original culture medium.

In normal biopsies maintained in culture for 24 hours, the morphology was well preserved and long villi with almost normal epithelium were seen There was little or no necrosis and many mitoses were present in the crypts. Electron microscopy confirmed that "in vitro culture for 24 hours maintained near normal morphology of the epithelial cells

In untreated coeliac disease the damaged intestinal mucosa improved strikingly during "in vitro culture for 24 or 48 hours. Light and electron microscopy showed that the abnormalities of the surface epithelial cells disappeared almost completely Moreover in some areas, short villi which were not present in uncultured biopsies, were visible after 24 hours of organ culture.

In cow s milk intolerance rapid changes appeared in intestinal mucosa cultured in a medium containing beta lactoglobulin and alpha lactalbumin provided that sufficient quantities of these milk proteins were added to the medium (300 gamma/ml of alpha-lactalbumin and 500 gamma/ml of beta-lactoglobulin)

These results suggest (1) that the harmful factors which contribute to maintaining the intestinal lesions "in vivo" in patients with coeliac disease are excluded in the in vitro culture system and (2) that cow s milk proteins have a direct toxic effect on the mucosa of intolerant children

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Mary A. Rossiter E. Ann Burgess & M. M. Libermann (London) *Unequal loss of sugar and peptide absorbing capacity by coeliac jejunal mucosa*

The well recognised fall in jejunal disaccharidase activity in coeliac disease could simply be ascribed to loss of microvillous surface area.

In jejunal biopsy specimens of children with gluten induced enteropathy mean levels of lactase maltase palatinase and sucrase were reduced to 0 07 0 15 0 17 and 0.14 of the mean normal values. However the glycyl leucine and glycyl glycine dipeptidase activities were only reduced to 0 54 and 0 72 of normal.

The uptake of ^{14}C labelled glucose and aminoacids has been measured by an in-vitro technique which is thought to represent active transport by the brush border In coeliac disease mean glucose uptake is reduced to 0 23 of that normally measured whereas leucine and glycine uptake are only reduced to 0 32 and 0 90 of normal.

There was good correlation of functions such as disaccharidase activity glucose uptake and leucine uptake with the degree of villous atrophy whether the cause was coeliac

disease or other forms of enteropathy such as postgastroenteritis malabsorption. Dipeptidase activity however varied from nil to normal in apparently similarly affected mucosae.

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serotyped according to the accepted international schema and both enteropathogenic and non-enteropathogenic serotypes were defined. Simultaneous cultures of E. coli from the faeces duodenum stomach and throat demonstrated that these organisms colonised the entire gastrointestinal tract

Certain E. coli serotypes are known to cause acute diarrhoea Our findings suggest that other E. coli serotypes may also be important in the aetiology of prolonged diarrhoeal illness. It is suggested that the presence of E. coli in the duodenum may be responsible for disturbances of carbohydrate and water transport found in such cases.

S Levin & M Mogilner (Rehovot) *Reye's syndrome (encephalopathy with fatty degeneration of viscera) due to warfarin poisoning*

The syndrome of acute encephalopathy and fatty degeneration of viscera was first described as an entity by Reye et al in 1963 It is characterised by rapid development of severe encephalopathy and acute liver failure often with fatal outcome Many aetiological causes have been ascribed to this disease including viral infections, poisons such as aflatoxins and drugs. Three cases are hereby reported in which warfarin (a derivative of 4-hydroxycoumarin) was the possible etiological agent.

R. Nelson (Birmingham) *Intractable diarrhoea of infancy treated by complete parenteral alimentation*

Eleven infants, under 12 months old received complete parenteral allimentation for intractable diarrhoea. The diarrhoea was due to a variety of causes All but one patient was severely underweight when first admitted. Weight loss continued in hospital as the diarrhoea prevented oral feeding Faecal testing for sugar and pH demonstrated only one case of carbohydrate intolerance.

Parenteral feeding was required for severe malnutrition. Seven children responded excellently by stopping diarrhoea within a week, and tolerating oral feeding within 3 weeks. Five tolerated an oral feeding formula which had produced severe diarrhoea before parenteral alimentation. In one infant diarrhoea finally ceased after 32 days, allowing satisfactory oral feeding. The child with carbohydrate intolerance, a secondary monosaccharide intolerance was unchanged after 50 days of complete parenteral feeding. Two infants died, of pneumonia secondary to a prolonged tracheostomy and of a primary immunological deficiency

In summary intractable diarrhoea characteristically affects young infants. These patients were underweight on admission, and continued to lose weight in hospital. Seven infants responded dramatically and 1 patient more slowly to treatment by complete parenteral feeding. These observations suggest that undernutrition may have contributed to the persisting diarrhoea of these children.

S Cadranel P Rodesch J P Butzler P Dekeyzer & H Loeb (Brussels) *Enteritis due to a "related vibrio An epidemiological study of 7 new cases in a nursery with isolation from stools and from gastric jejunal and ileal juices*

Though diarrhoea was a constant symptom in the 14 observations after the first human cases were described by E. O King, only recently Dekeyzer et al. isolated the related vibrio not only from the blood but also from the stools of a young adult female with gastrointestinal symptoms. In the same laboratory Butzler et al found the related vibrio in 45 out of 900 stools of children and adults with diarrhoea the vibrio has also been found 13 times in stools of 1 000 symptom-free children

The authors report a case of a 16-month-old boy admitted for failure to thrive and diarrhoea which had begun 2 months earlier when the child was admitted to a nursery

From numerous stool cultures, negative for the usual pathogens, a related vibrio was constantly isolated. The course of the infection was protracted and three treatments with tetracyclines and with neomycin brought about transient normalization of the stool cultures. Reappearance of the related vibrio in the stools were concomitant with relapses of the diarrhoea. During each course of antibiotic treatment, the gain in weight was significant.

At the same time, an outbreak of enteritis occurred in the nursery and the same related vibrio was found only in the stools of children with symptoms. Gastric, jejunal and ileal juices were obtained for culture. In several instances the related vibrio was found at more than one level of the digestive tract.

A new device was used which allows simultaneous collection of the secretions without contamination from the upper digestive and respiratory tracts, as confirmed in vitro according to the procedure of Shiner et al.

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A. G Marchi, F Tuvo & S. Nordio (Trieste, Genoa): *Child autism and gluten intolerance*

Dohan drew attention to a possible relationship between coeliac disease, schizophrenia and, perhaps, childhood autism. He emphasized that these may be polygenic diseases having some genes in common.

We studied two siblings who seem to confirm such a hypothesis. Paolo became severely anorexic and set himself more and apart from the environment when he was 20 months old. Diagnosis of child autism was made according to Creak's criteria. Cerebral electric activity was poorly organized and the EMG and the study of maximal nervous conduction velocity showed cerebral immaturity and some degree of denervation, as in some metabolic diseases and toxic peripheral neuropathies. The intestinal mucosa was flat. A xylose test gave abnormal results and intestinal malabsorption of fat was demonstrated. An oral tryptophan load provoked increased elimination of xanthurenic acid and the faeces contained abnormal indole metabolites.

A gluten-free diet led to a very evident amelioration of behaviour disturbances, nervous abnormalities (EEG EMG etc.) and intestinal dysfunction.

The sister (Roberta) when 16 months old, showed a very similar behaviour disturbance. As a consequence of the surroundings she slowly improved up to apparently complete normality The intestinal mucosa was also flat. The father suffered from diabetes, and a paternal aunt of these patients had suffered from severe anorexia in childhood. A diagnosis of anorexia nervosa was made.

S. Guandalini, A. Rubino & S. Auricchio (Naples): *Intestinal transport of dipeptides by newborn rabbits*

The transfer of dipeptides from the lumen into the intestinal mucosa of adult rabbits is mediated by at least one transport system selective for dipeptides and unshared by amino acids free in the lumen (1). In the present study this intestinal transport mechanism for dipeptides has been investigated, *in vitro* in 1 to 6-day-old rabbits. The unidirectional influxes of glycine from mediums containing either (^{14}C-Gly) glycyl-L-proline (Gly-Pro influx) or ^{3}H-glycine (*Gly influx*) were measured by incubating segments of small intestine under conditions essentially similar to those described previously (1).

In the ileum of newborn animals, Gly-Pro influx follows a Michaelis–Menten type of kinetics with an affinity constant (K_t) of 0.53 mM and a maximal velocity (V_{max}) of 50.6 µmoles/g tissue h. Removal of sodium from

serotyped according to the accepted international schema and both enteropathogenic and non-enteropathogenic serotypes were defined Simultaneous cultures of E. coli from the faeces duodenum stomach and throat demonstrated that these organisms colonised the entire gastrointestinal tract

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to serum levels of FFA and glycerol under various conditions, several authors have discussed the possibility that the obese state is associated with a defect in fat mobilization. Though these abnormalities could be established only over a short period of fasting or by administration of lipolytic agents in pharmacological doses, such an assumption seemed to be confirmed by observations indicating resistance to ketosis in obese adults. In this respect some individual differences may really exist. However it seems unlikely that even overweight individuals are able to tolerate prolonged fasting without mobilizing the energy resources of their fat stores, especially childhood.

The moment at which there was a significant rise in serum FFA, glycerol and ketone bodies during a one-week period of starvation was found by estimating these parameters daily in six obese children Furthermore, our investigations were performed to clarify to what extent ketogenesis occurs and whether there is any relation between the rise in FFA and glycerol and the development of ketosis.

Our results can be summarized as follows: In spite of some individual differences after the first day of starvation there was only a relatively small rise in all parameters, and this was independent of the initial serum levels during normal food intake. A marked rise in FFA and glycerol was observed in half the probands on the third day of starvation, and in the others on the fourth day In nearly all the obese children the ketone bodies increased markedly at exactly the same time. The blood concentrations of beta-hydroxybutyrate increased four to eleven-fold (mean seven-fold) on the fourth day Following this peak the serum levels of both FFA and glycerol showed a transient decrease, then rose again about the sixth day of starvation. The blood ketones followed a similar and simultaneous trend.

From these findings in obese children we can conclude that there is in fact a diminished response in all parameters during the first two days of starvation, when compared with normal weight individuals. However about the third and fourth day of fasting obviously an *overflowing* lipolysis causes an excessive rise in serum levels of FFA, glycerol and ketone bodies indicating that there is no resistance to ketosis in obese children *per se*

J A. Dodge (Cardiff): *The genetics of infantile pyloric stenosis*

A study of infantile pyloric stenosis was conducted in Belfast, involving 480 index patients. This was a 91.3% follow up of all surgically treated cases during a 20-year period. Twin data showed that identical twins were not always concordant for pyloric stenosis, and also suggested that asymptomatic cases could occur The findings in first second and third degree relatives supported Carter's evidence that male relatives of female index patients are particularly at risk. This is compatible with a polygenic mode of inheritance and a differential sex threshold.

One of the genetic factors involved may be the ABO blood group of the patient. In this series, there was an abnormal blood group distribution with a marked excess of groups O and B relative to group A. Secretor status was similar to that of the normal population.

T Lücking & R. Grüttner (Hamburg): *Chronic diarrhoea and severe malabsorption in infancy following infections with pathogenic E coli*

While coeliac disease is a well-defined intestinal disorder the aetiology of malabsorption remains uncertain in a number of children. We studied a group of 7 infants whose disease appeared to be related to intestinal infections. Pathogenic E. coli strains were detected in stool cultures at the onset or during the course of the disease. Four of these patients became ill at ages of 2 to 5 weeks, and 3 at ages of 3 to 9 months. They suffered from severe

the incubation medium results in a significant reduction of V_{max} while K_t is unchanged Influx from 0.5 mM Gly Pro is not affected when glycine L-phenylalanine L-leucine L glutamic acid or L lysine are included in the incubation medium at 20 mM concentrations, while it is inhibited by more than 90* in presence of 5 mM L-leucyl L leucine or 5 mM L-alanyl L proline

Influx from 0 5 mM Gly Pro is 41 9±3 5 (μmoles/g tissue h mean ±S E.M) for the proximal jejunum 20 7±2.1 for the distal ileum Control values obtained with adult rabbits are 3 8±0 4 and 6 0±0 6 respectively In newborn animals Gly influx from 0.5 mM Gly is 3 3±0 1 for the proximal jejunum and 5 7±1 1 for the distal ileum

It is concluded that in the small bowel of newborn rabbits, the translocation process for the glycine residue of glycyl L-proline shows properties fundamentally similar to those described previously for adult animals, but the maximal velocity is much higher in newborn Due to this transport process for dipeptides the intestinal uptake of glycine is much larger when this glycine is presented to the mucosa in form of glycyl L proline than when it is presented in the free form This advantage of the Gly residue of Gly Pro as compared to free Gly is more accentuated in jejunum than in ileum

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J Schmitz, F Rey & J Rey (Paris) *Perfusion study of absorption of monosaccharides and alpha-glucosides in children*

Previous perfusion studies in adults, focused on the relationships between hydrolysis of alpha-glucosides and absorption of monosaccharides, tend to confirm Crane's model of the organization of the intestinal brush border Further investigation of whether these relations were or were not affected by the anatomical condition of the mucosa was of interest both from a theoretical point of view and with regard to the treatment of fermentative diarrhoea.

Nine children whose intestinal mucosa was either normal (4) flat (coeliacs, 3) or hypertrophic (secondary to important small bowel resection 2) were perfused with a double lumen tube five randomized loads of glucose fructose sucrose or maltose were infused P E.G being used as a non-absorbable marker Total sugars, reducing sugars, glucose and P E.G were assayed.

Glucose absorption and sucrose and maltose hydrolysis were found to follow identical saturation curves in the three groups of children In every case the maximum velocity was lowest in the coeliacs, and highest in the children with small bowel resection, whereas the half saturating load was found to be constant for each substrate and common to the three of them 100% of the glucose and 70% of the fructose released by sucrose hydrolysis were absorbed 75% of the glucose released by hydrolysis of maltose was absorbed The absorption of fructose was linear and was lowest in the coeliacs and highest in the resected children.

These results suggest a very close relationship between the sucrose protein and the glucose carrier glucose transfer being the rate-limiting step whatever the anatomical state of the mucosa They show clearly that in coeliac disease the alteration of the digestive-absorptive capacity is a consequence of the diminished number of active sites. Finally they seem to show that there is no theoretical advantage in giving the constitutive monosaccharides of sucrose and/or maltose instead of these disaccharides in malabsorption syndromes with fermentative diarrhoea.

U Spahn I. Hobert W Plenert & E. Petrich (Jena) *Ketosis in obese children during starvation*

Since there is some evidence that obese and normal-weight individuals differ with regard

sured. Serum immunoglobulin, serum iron, serum folate, A.B.O blood groups, colour vision, ability to taste phenylthiourea and fingerprint patterns were also measured. A slight excess of ridge-shaped villi was noted in the jejunal mucosae of the relatives, but, although this was significant at a 0.02 level it was not possible to exclude observer bias. 7 of 37 coeliac relatives with mucosal jejunal biopsies were found positive for reticulin antibodies while 20 controls tested were all negative. No other difference was noted between the relatives and controls.

The incidence of coeliac disease in the west of Ireland is greater than 1 in 448. The incidence of other coeliacs in families in which there is already 1 coeliac patient is 10%

H. Loeb, A. van Steirteghem & M. Bossuyt (Brussels): *Jejunal mucosa in infantile marastic kwashiorkor*

Data concerning the aspect of the intestinal mucosa of children with protein or protein calorie malnutrition are scarce and sometimes contradictory (1–9)

The present tests were performed in 12 children of the Bashi tribe in the Paediatric Hospital Centre of Lwiro in the Kivu area of the Republic of Zaire. All the children, aged 2 to 4 years, had protein-calorie malnutrition with hypoproteinaemia, in 2 of them untreated and in the others after 2 to 17 weeks of refeeding.

Intestinal biopsies with the paediatric Crosby capsule were taken without fluoroscopic control. Only one sample was from the duodenum, the others showed characteristic jejunal structures. There were no complications.

At direct examination of the biopsies, aspects of leaves and ridges were the features most often encountered, flat mucosa was not seen.

At histological study only minor villous atrophy could be found (grades II and III), except in one case of generalized oedema with hypoproteinaemia, in which subtotal villous atrophy (grade IV) was found.

These results are in agreement with the observations reported in the recent literature concerning infantile marastic kwashiorkor. There is apparently no correlation between the degree of villous atrophy and the severity of the clinical picture the duration of treatment or the level of serum proteins.

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D Kaiser U Axmacher & E. Drack (Berne): *Micropuncture of single ileal villi in the adult and newborn rabbit*

A micropuncture technique has been developed to study the formation and electrolyte composition of primary lymphoid fluid in the central lacteal of an ileal villus. Small pieces of rabbit ileal mucosa were incubated in Krebs solution and the central lacteal of a single villus was punctured under a stereo microscope. By injecting coloured paraffin oil the lower part of the duct was blocked, and a second capillary was inserted into the upper part of the villus for the collection of samples.

The following results were obtained.

1 The reabsorbing capacity of a single villus ranges between 0 10 and 1.5 nanoliter/min when the surrounding medium is isotonic with plasma.

2. The sodium concentrations are slightly above the plasma values, whatever the tonicity of the luminal fluid may be.

3 The potassium concentrations reach 7.5

recurrent or chronic diarrhoea with frequent and life threatening episodes of precipitous weight loss These infants developed a chronic malabsorptive state which was refractory to various attempts at dietary management and in some necessitated parenteral nutrition for several weeks.

It was shown by loading tests or analysis of the response to dietary manipulations that the persistence of diarrhoea was due to malabsorption of sugars. All infants had malabsorption of lactose 2 possibly had additional malabsorption of sucrose and one was unable to absorb fructose In 3 children intestinal biopsies were obtained during the active phase of the disease Two of them showed flattened convolutions with decreased disaccharidase activities while the third infant had a normal mucosa.

Elimination of the offending sugar(s) from the diet was followed by clinical improvement Follow-up studies during remission revealed neither disturbances of absorption nor histological abnormalities of the mucosa.

C. Ricour & J Rey (Paris) *Kinetics of fat hydrolysis and micellar solubilization in children with pancreatic deficiency*

Quantitative measurements of external pancreatic secretion have been done in children during pancreozymin secretin stimulation The percentages of hydrolysis and micellar solubilization have also been determined in adults after a test meal The correlation between the different parameters and the type of pancreatic deficiency have not as yet been studied.

A constant rate lipid intestinal perfusion technique (20 mg/mn/m²) was used in 6 children 3 of whom suffered from cystic fibrosis of the pancreas (CF) (one without steatorrhoea) 2 from congenital pancreatic hypoplasia (PH) and 1 from isolated lipase deficiency (LD) The composition of the oil and micellar phases, lipase activity concentration of bile salts and intraluminal pH were measured according to the techniques previously described.

A sharp decrease in hydrolysis to below 10% ($N=50$–60%) and in FFA solubilization to below 20% ($N=35\pm8$) after 180 min were found in CF however MG solubilization was normal In LD and PH hydrolysis was also reduced considerably but solubilization of the polar lipids was normal after the 60th mn 60% of the FFA and 80% of the MG ($N=40\pm9$) were concentrated in the micellar phase identical results were observed in one case of CF with subnormal intraluminal pH. In the case of CF without steatorrhoea, the only change was a slowing down of FFA solubilization which with the low pH, points to an isolated disturbance secretion. Hydrolysis and/or solubilization defects can be corrected by adding pancreatic extract or a phosphate buffer pH 6 7 to the infusion.

M J Mylotte B Egan-Mitchell, P F Fottrell, C. F McCarthy & B McNicholl (Galway) *Familial coeliac disease*

From a study of the number of childhood and adult coeliacs presenting to the Regional Hospital, Galway the incidence of coeliac disease was determined to be 1 in 448.

The first-degree relatives of 29 index patients with coeliac disease were studiend. At the beginning of the investigation, these 29 index patients were known to have 3 siblings with coeliac disease. 120 other first-degree relatives had jejunal biopsies performed and in 12 the mucosa showed subtotal villous atrophy Seven of the 12 with flat mucosal biopsies were in 2 families. The jejunal mucosae of the relatives who did not have coeliac disease were compared with those from a group of volunteer controls and with jejunal biopsies from hospital patients who were undergoing gastrointestinal investigation. The mucosa was examined histologically and with the dissecting microscope. In selected instances, electron microscopy was done. Peptidase and disaccharidase levels were meas-

ured. Serum immunoglobulin serum iron, serum folate, A.B.O blood groups, colour vision, ability to taste phenylthiourea and fingerprint patterns were also measured. A slight excess of ridge-shaped villi was noted in the jejunal mucosae of the relatives, but, although this was significant at a 0.02 level, it was not possible to exclude observer bias. 7 of 37 coeliac relatives with mucosal jejunal biopsies were found positive for reticulin antibodies while 20 controls tested were all negative. No other difference was noted between the relatives and controls.

The incidence of coeliac disease in the west of Ireland is *greater than 1 in 448*. The incidence of other coeliacs in families in which there is already 1 coeliac patient is 10%.

H. Loeb A. van Steirteghem & M. Bossuyt (Brussels): *Jejunal mucosa in infantile marastic kwashiorkor*

Data concerning the aspect of the intestinal mucosa of children with protein or protein-calorie malnutrition are scarce and sometimes contradictory (1–9).

The present tests were performed in 12 children of the Bashi tribe in the Paediatric Hospital Centre of Lwiro in the Kivu area of the Republic of Zaire. All the children, aged 2 to 4 years, had protein-calorie malnutrition with hypoproteinaemia, in 2 of them untreated and in the others after 2 to 17 weeks of refeeding.

Intestinal biopsies with the paediatric Crosby capsule were taken without fluoroscopic control. Only one sample was from the duodenum the others showed characteristic jejunal structures. There were no complications.

At direct examination of the biopsies, aspects of leaves and ridges were the features most often encountered, flat mucosa was not seen.

At histological study only minor villous atrophy could be found (grades II and III) except in one case of generalized oedema with hypoproteinaemia, in which subtotal villous atrophy (grade IV) was found.

These results are in agreement with the observations reported in the recent literature concerning infantile marastic kwashiorkor. There is apparently no correlation between the degree of villous atrophy and the severity of the clinical picture the duration of treatment or the level of serum proteins.

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D Kaiser U Axmacher & E. Drack (Berne): *Micropuncture of single ileal villi in the adult and newborn rabbit*

A micropuncture technique has been developed to study the formation and electrolyte composition of primary lymphoid fluid in the central lacteal of an ileal villus. Small pieces of rabbit ileal mucosa were incubated in Krebs solution and the central lacteal of a single villus was punctured under a stereo microscope. By injecting coloured paraffin oil the lower part of the duct was blocked, and a second capillary was inserted into the upper part of the villus for the collection of samples.

The following results were obtained

1 The reabsorbing capacity of a single villus ranges between 0.10 and 1.5 nanoliter/min when the surrounding medium is isotonic with plasma.

2 The sodium concentrations are slightly above the plasma values, whatever the tonicity of the luminal fluid may be.

3 The potassium concentrations reach 7.5

mEq/l 1 compared with 5 0 in normal plasma

4 Ouabain at the concentration of 10^{-4} mol and ethacrinic acid at 10^{-3} mol markedly reduce the flow without changing the ionic composition

5 The rate of fluid formed closely depends on the presence of sodium in the bathing fluid

E. Blanche Butler & A. Holzel (Manchester) *Cytochemistry of gastric epithelium in the neonate and infant*

In view of the very limited knowledge of the mucosal changes in gastric disorders of infancy an attempt has been made to establish an in vitro method of investigation which is reliable and easily reproducible Fragments of gastric mucosa obtained by gastric washing are concentrated and processed histologically Serial sections through the block are stained for mucopolysaccharides using a modification of the methods described by Spicer et al (2) Technical problems prevented the use of cryostat sections from the cell block to demonstrate enzyme reactions in gastric epithelium. The gastric wash was used with the usual substrates and incubation times to produce a colour reaction in solution. The methods of Planteydt & Willighagen (1) were used

1 *Mucopolysaccharides.* Most infants at birth show neutral and acid non sulphated mucopolysaccharides but in a group who showed only neutral mucopolysaccharides (adult pattern) the baby was more likely to be seriously ill.

2. *Enzyme reactions* The results in the gastric wash did not correspond with the results of Planteydt & Willighagen (1) on adult stomach No references could be found to similar work in the neonate but we were able to examine enzyme reactions of gastric epithelium in two fresh necrospy specimens. Some of the differences in the wash could be due to contamination with milk and/or polymorphs. Tests on milk showed variation of the enzyme pattern between human milk cows mild and "filled in milk There were some similarities between enzyme patterns in solution and sections from an infant of 35 weeks gestation; the most interesting of these were a positive reaction to LAP and a negative reaction to isocitric dehydrogenase

References

1 Planteydt H. T & Willighagen R. T G *J Path Bact 80* 317 1960.
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H. Götze J W Adelson & B Hadorn (Berne): *Hormone-controlled small intestinal enzyme release*

Three intestinal hydrolases, enterokinase, sucrase and alkaline phosphatase were released into the perfused intestinal lumen of the rat after intravenous injection of the hormones cholecystokinin pankreozymin (CCK PZ) and gastrin. This release was further augmented by the presence of bile in the perfusate. In vitro-addition of bile led an activation of hormonally liberated enterokinase, but had no effect on sucrase and alkaline phosphatase activity The three enzymes differed in their sedimentability after ultracentrifugation (113 000 *g*) enterokinase remained almost completely in the supernatant fluid, 80–90% of the sucrase and alkaline phosphatase were sedimentable The data suggest that intestinal enzyme release is hormonally controlled although the mechanism of release differs in the three enzymes which were examined

References

1 Gotze H., Adelson, J W Hadorn H. B Portman R. & Troesch V *Gut* in press, 1972.

K. H. Niessen K Schmidt & G Brüggmann (Tubingen) *Secondary deficiency of enterokinase in the duodenal mucosa associated with partial and total atrophy of the villi of the duodenum*

Biopsies of the duodenum were assessed in 31 children aged 3 months to 14 years, with respect to histology activity of disaccharidases and enterokinase.

Three groups could be distinguished by histological criteria. 10 children revealed normal duodenal mucosa, 14 showed partial atrophy of the villi, and 7 had absence of villi. A semiquantitative key system was constructed, which arranges the histological findings as follows: villi and crypts are classified according to their length, the stroma by the extent of cell infiltration and the epithelium by the content of goblet cells.

Disaccharidases were estimated according to the method of Dahlqvist in the homogenate. The activity of enterokinase in the homogenate was evaluated by a modification of Hadorn s method, in which the trypsin split off from a trypsinogen solution is measured, with BAPNA as indicator One unit of enterokinase was defined as the amount of enzyme that liberated 1/μg of trypsin in 1 min under the conditions of the test. Normal range: $x = 17\ 68$ EKU/mg, $s = 3.66$ EKU/mg. The severity of the mucosal changes was reflected in the decreased activity of disaccharidases. In cases of partial as well as complete atrophy of the villi enterokinase activity was generally reduced by about 50% compared with the control group. We see here a parallel with the behaviour of most dipeptidases, which also show reduction of activity in coeliac disease although less markedly than the disaccharidases. It is supposed that the reduction of enterokinase leads to a diminution of trypsin in the duodenal juice and consequently reduced digestion of protein.

G Zoppi, G Andreotti, F Pajno & D Gaburro (Verona, Ferrara): *The fluid protein and electrolyte contents and outputs in duodenal juice before and after stimulation with pancreozymin and secretin in full-term and premature newborn infants*

With a specially manufactured duodenal microtube which permits easy and rapid intubation of the newborn, we studied pancreatic production of fluid, protein and electrolytes before and after stimulation with pancreozymin and secretin in 12 full-term and 22 premature newborn infants.

In each subject, we performed three pancreozymin-secretin tests: at birth, before the first feed 24 hours after and 1 week after birth. The results, reported as secretion rates per kg body weight per minute show that in duodenal juice.

(1) the rates of secretion of fluid, protein, Na K Cl HCO_3 and Ca are, with a few exceptions higher in premature than in full-term newborn infants,

(2) the increase in the rates of secretion of fluid, protein and electrolytes after the hormonal stimulations is higher at one week than at birth and this difference is more evident in premature than in full-term newborn infants,

(3) the difference between the pancreozymin and secretin responses, which is well known in older children and in adults, is not present at birth but appears at 1 week of age.

The results, which complete our research on exocrine pancreatic function in full-term and premature newborn infants, besides showing secretion patterns similar to those already observed for the enzymes, indicate that the exocrine pancreas does not specifically recognize the pancreozymin and secretin stimuli at birth.

S. Levin, Sara Goren & E. Fishel (Rehovot) *Soft radiological signs in the diagnosis of duodenal ulcer disease in children*

It is well known that the younger the patient, the more difficult it is for the radiologist to demonstrate an actual duodenal ulcer The more perseverent and understanding the radiologist, the higher the incidence of radiological diagnosis. However in many

mEq/l 1 compared with 5 0 in normal plasma

4 Ouabain at the concentration of 10^{-4} mol and ethacrinic acid at 10^{-3} mol markedly reduce the flow without changing the ionic composition

5 The rate of fluid formed closely depends on the presence of sodium in the bathing fluid

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References

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Acta Pædiat Scand 63 173 1974

PROCEEDINGS OF PAEDIATRIC SOCIETIES

THE FINNISH PAEDIATRIC SOCIETY

MEETING IN HELSINKI, FEBRUARY 17 1973

Bent Friis-Hansen (Copenhagen, Denmark): *Oxygen therapy of the newborn*

The aim of oxygen therapy in the newborn period is to balance the supply of oxygen to the tissues with their requirements. The oxygen supply is dependent on the processes of 1) ventilation, 2) diffusion of O_2 from the alveoli into the blood, 3) pulmonary circulation, especially right-to-left shunts, and 4) the O_2-carrying capacity of blood, determined by the concentration and O_2 dissociation curve of hemoglobin and the rate of perfusion of the tissues. The O_2 requirements of the organism are affected by temperature, and among other factors, by the amount of work needed for respiration.

Right-to-left shunting, both intrapulmonary by circulation through unventilated areas and through foramen ovale and the ductus arteriosus, determines the extent to which Pa_{O_2} can be increased by elevating the O_2 concentration of inspired air If a 50% shunt is present, even 100% O_2 will not elevate Pa_{O_2} above 46 mmHg. Shunting has a much smaller effect on P_{CO_2}. Acidosis is a major factor in creasing pulmonary vascular resistance and right-to-left shunt, and hypoxia has a similar effect In order to maintain adequate pulmonary circulation, Pa_{O_2} should be kept between 50 mmHg and 75 mmHg and pH above 7.30. Higher oxygen tension increases the risk of retrolental fibroplasia of the eyes.

Of the various hypotheses about the etiology of the respiratory distress syndrome (RDS), lack of surfactant lining the alveoli seems the most plausible today The following pathogenic sequence can be pictured. Immaturity associated with delayed development of surfactant leads to incomplete lung expansion after birth and to low alveolar ventilation, resulting in hypoxia and acidosis despite increased respiratory efforts. Right-to-left shunt increases, a shock-like state ensues and pulmonary capillary permeability is increased, leading to exudation of fluid into the alveoli atelectasis and hyaline membranes. In this vicious circle O_2 and bicarbonate therapy combats the hypoxia and acidosis, and improves pulmonary circulation which enhances surfactant synthesis. Intermittent positive pressure ventilation (IPPV) improves lung expansion and alveolar ventilation, decreases respiratory work and the attendant increased energy needs, and counteracts atelectasis.

Continuous positive airway pressure (CPAP) represents a major advance in the treatment of RDS. By preventing alveolar collapse in surfactant-poor lungs and exudation of fluid, CPAP has a good effect on Pa_{O_2}, but less so on P_{CO_2} or pH However only about one half of patients with RDS get along with CPAP alone while the rest still require IPPV Since the addition of CPAP to the therapeutic arsenal at our hospital, the mortality of RDS in infants weighing 1 500–2 000 g has decreased from about 75% to 25% A smaller benefit has been observed in babies weighing over 2 000 g, whereas in those below 1 500 g the method has had little effect on the 90% mortality

The indications for initiating CPAP depend mainly on the clinical picture, but the following laboratory values serve as guidelines: Pa_{O_2} below 50 mmHg (in 100% O_2), P_{CO_2} above 60 mmHg and pH below 7.20. If Pa_{O_2} falls below 40 or P_{CO_2} rises above 70 IPPV is indicated.

Kari Raivio

cases of children of all ages suspected of having a peptic ulcer only "soft radiological signs may be found These are nonspecific secondary manifestations such as (*a*) irritability of the duodenal bulb (*b*) deformity of the bulb (*c*) rapid early emptying of the stomach with delayed late emptying (*d*) increased mucosal markings and (*e*) increased secretion of gastric juice

We have compared two groups of children presenting with a clinical picture compatible with peptic ulcer disease. In one roentgen examination demonstrated an actual ulcer and in the second group, only "soft" roentgenological signs were present. No significant differences could be found in the 60 children studied with regards to clinical picture, etiological factors, genetic factors and response to "ulcer regime" therapy It is suggested that more significance should be placed on the non-specific, compatible radiological signs in the diagnosis and treatment of duodenal ulcer disease in children.

Pekka Kuitunen

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Kari Raivio

NEW BOOKS RECEIVED

K. Diebold *Die erblichen myoklonisch-epileptisch dementiellen Kernsyndrome Progressive Myoklonusepilepsien Dyssynergia cerebellaris myoclonica myoklonische Varianten der drei nachinfantilen Formen der amaurotischen Idiotie* 254 pp. illus. Hippius, Janzarik & Müller (eds.): Monographien aus dem Gesamtgebiete der Psychiatrie Psychiatry Series, Band 8 Springer Verlag, Berlin, Heidelberg, New York 1973 US $36.30

M Winick (ed): *Nutrition and development* 245 pp., illus. John Wiley & Sons New York London Sydney Toronto 1972. £7.80

L. M Solomon & N B Esterly: *Neonatal dermatology* 214 pp illus. A J Schaffer (ed): Major problems in clinical pediatrics, vol. IX W B Saunders Company Philadelphia, London, Toronto 1973 £7 05

G Seifert (ed): *Verhandlungen der Deutschen Gesellschaft für Pathologie 56 Tagung vom 16 bis 20 Mai 1972 in Graz*, 749 pp., illus. Gustav Fischer Verlag Stuttgart 1972 DM 242 —

H Bossart, J M Cruz, A Huber L. S Prod'hom, J Sistek (eds): *Perinatal Medicine* Third European Congress of Perinatal Medicine, Lausanne April 1972 399 pp., illus Hans Huber Bern Stuttgart, Wien 1973 DM 88 —

Jerome Beker (ed): *Critical incidents in child care* A case book for child care workers, 375 pp Behavioral Publications, New York 1972. $7.95 soft, $15 95 hard

D W Smith & A A Wilson. *The child with Down's syndrome (Mongolism)* 106 pp illus. W B. Saunders Company Ltd Philadelphia London Toronto 1973 £2.45

J C. Somogyi (ed.): *Nutrition and technology of foods for growing humans* 288 pp., illus. Proceedings of the Symposium of the International Union of Nutritional Sciences, the International Union of Food Science and Technology and the Institute for Nutrition Research, Zürich, October 1971 Series of the Institute of Nutrition Research, vol. 18. S Karger AG Basel 1973 DM 84 —

H J Kaufmann (ed) *Intrinsic diseases of bones*, 594 pp. illus. H J Kaufmann (ed.): Progress in Pediatric Radiology vol. 4 S Karger AG Basel 1973 DM 190 —

P S Timiras. *Developmental physiology and aging* 692 pp., illus Collier Macmillan Publishers, London 1973 £9.95

G H Weber & B J Haberlein (eds.): *Residential treatment of emotionally disturbed children* 377 pp Behavioral Publications, New York 1972. $14.95.

Youth and drugs World Health Organization Technical Report Series No. 516. Geneva 1973

BOOK REVIEWS

N. B. Talbot, J. Kagan & L. Eisenberg (eds.): *Behavioral science in pediatric medicine.* W. B. Saunders Company LTD Philadelphia, London, Toronto 1971 467 pp. £7.25.

In the preface the editors say "Current clinical experience with pediatric patients indicates that behavioral and social disorders are no less important than major physical and biological agents as causes of disease and disability among infants, children and adolescents" Therefore it has been their intention to give special information of the behavioural sciences to "students and practitioners of pediatric medicine and allied health professions"

Thirteen authors from universities and medical schools in the United States have written 9 chapters, most of them dealing with different kinds of human behaviour

The first chapter by N. B. Talbot and Mary Howell is on "Social and Behavioral Causes and Consequences of Disease among Children" Their description of psychosocial factors in disease among children is valuable and gives a structured and systematic approach which can be used in practice. They emphasize the psychosocial supplies which are of fundamental importance for every child especially during the first years of life. They have several valuable recommendations about the diagnosis of psychosocial deprivation but of course it is more difficult to find useful therapeutic strategies. They recommend parental education and social service for the families. But it is of course difficult to change attitudes and patterns as anyone working with these problems knows.

Chapters two and three deal with "Physiological Psychology" and "Perception" Both are rather theoretical and of little interest to Scandinavian readers. The following chapter on "Children's Learning" by S. H. White and H. D. Fishbein describes different learning theories and their relation to human adaptation. Piaget's theory of cognitive development is referred to and its importance to understand behaviour is emphasized Furthermore, infant–mother interaction is described according to Escalona, noting that it is a mutual organization of the mother's and child's behaviour In this chapter there is also a short description of various forms of behaviour modification. This part is informative for the pediatrician.

One of the editors, J. Kagan, has written about "Personality Development" The author discusses in this chapter Piaget's important theories and describes the concept of identification, the role of the family and the mother-child interaction. Certainly there is some overlapping of the text in relation to the foregoing parts. Nevertheless, the whole chapter is well worth reading.

A comprehensive report on "Childhood Accidents and Injuries" with much statistical data and analysis of injuries and their causes is given in another chapter The prevention of injuries is discussed. Lead poisoning is given as an example of a problem which can be eliminated but still exists in the US. In the Scandinavian countries this type of poisoning belongs to history

The last chapter is written by L. Eisenberg and C. K. Conners and deals with "Psychopharmacology in Childhood" The task to write about drug treatment of behavioural disorders in children is not easy On the whole it is doubtful if one can give a satisfactory description on this subject because of lack of adequate reports on the use of psychopharmaca in children. Stimulant drugs such as amphetamine are explicitly discussed. However these drugs cannot be prescribed in Sweden because of the abuse among adolescents and adults. The authors consider that such risk of addiction among children has not yet arisen.

Each chapter ends with a comprehensive reference list and the last 40 pages contain a valuable index. The whole volume can be recommended and is a useful contribution to diagnosis and treatment in pediatric medicine.

Lennart Sonesson

T. Baumann & R. Frey: *Epilepsie im Kindesalter* 2nd ed., Pädiatrische Fortbildungskurse für die Praxis, Vol. 26 (E. Rossi ed.) S. Karger Basel 1972, 140 pp. sFr 39.—

This book is a rewritten report from a postgraduate course and a rather up-to-date survey of the essential of epilepsy in childhood. At the same time it is a short, easy to read and entertaining book.

Doose has written a most interesting chapter on the genetics of epilepsy essentially dealing with his own investigations. Dumermuth and Scollo-Lavizzari have succeeded in giving a very comprehensible concept of the pathogenesis of epilepsy Of greatest interest, however from a clinical point of view is Kruse's very well written chapter on therapy A comparing discourse on infantile spasm and akinetic-myoclonic seizures is stimulating but seems beyond the scope of a book like this. The authors (and others) way of using personal names for terminology is rightly reproved elsewhere in the book. Other chapters deal with symptomatic epilepsy epilepsy caused by metabolic disorders, social and psychological problems and adverse drug reactions.

This book is well worth reading by pediatricians but is too basic for specialists of pediatric neurology

Örjan Johansson

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NUTRITION IN LOW-BIRTH WEIGHT INFANTS

II *Repeated Intravenous Injections of Fat Emulsion*

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ABSTRACT Gustafson, A., Kjellmer I., Olegård, R. and Victorin L. (Department of Paediatrics, Children s Hospital, and of Medicine I, Sahlgren's Hospital Göteborg, Sweden). Nutrition in low-birth-weight infants. II. Repeated intravenous injections of fat emulsion. Acta Paediat Scand 63: 177 1974.—The elimination of an exogenous fat emulsion from the blood stream after repeated intravenous injections was investigated in two groups of low-birth-weight infants: 11 appropriate-for-date (AFD) pre-term babies and 8 light-for-date (LFD) pre- and full-term infants. During a period with six injections hourly of 0.15 g fat/kg b w the total lipids of plasma increased only moderately in the AFD group from 264 to 351 mg/100 ml, while in the LFD group a progressive rise of total lipids occurred from 244 to 466 mg/100 ml. The plasma turbidity increased correspondingly more in the LFD than in the AFD group. In 5 LFD babies, where a progressive accumulation of total lipids occurred with each injection of fat emulsion, heparin was given intravenously after eight fat injections. The plasma was rapidly cleared of fat although fat injections were continued. It is concluded that AFD infants are able to hydrolyse fat emulsions given at an hourly rate of 0.15 g/kg b.w., while this amount of fat to LFD babies will cause an accumulation of plasma lipids unless heparin is supplied simultaneously

KEY WORDS: Low-birth-weight infants, nutrition, fat emulsion, parenteral feeding

The need for an adequate supply of calories during the neonatal period in low birth weight infants has been documented in several ways (1 3) As a first attempt to increase the caloric content in our parenteral program we studied the elimination of a single intravenous dose of fat emulsion Intralipid® That study (7) demonstrated a calculated maximal removal capacity which averaged 0 3 g fat/(kg hr) in pre-term infants with birth weights appropriate for gestational age (AFD). The babies were studied during their first 24 hours of life be infants who were light-for-date (LFD) two features were observed These babies had a reduced capacity to eliminate fat and they demonstrated a particular response to a fat load in their lipoprotein pattern with a marked increase in the pre-beta-lipoprotein fraction

The present study was designed to study the elimination of fat in these two groups of patients when Intralipid® was supplied over several hours at a rate corresponding to half the maximum removal capacity i e 0 15 g/(kg hr) During the course of the study it became obvious that the LFD infants cleared their plasma of fat at a significantly lower rate than the AFD babies and that those in the LFD group instead accumulated gradually increasing concentrations of fat in their plasma.

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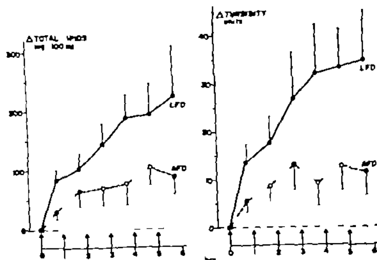


Fig 2 Changes of (Δ) plasma total lipids and turbidity from the initial level during the first 6 hours of fat administration. Mean ± S.E.M. are given. ●—● LFD group. O- -O AFD group. Arrows indicate fat injections.

same in the AFD group (264±22 *mg/100 ml*[1]) as in the LFD group (244±30 *mg/100 ml*). Forty minutes after the first injection a difference was observed between the two groups the LFD group reaching higher values ($p<0.01$). This difference became more pronounced with additional injections of fat emulsion. Figure 2 demonstrates the increase of total lipids from initial pre-injectional level. Corresponding changes in plasma turbidity are also included. The pre-injectional absolute values for turbidity are slightly different (AFD: 6.5±0.8. LFD: 8.6±1.5 Units respectively) but the difference was not statistically significant ($p>0.05$). All values after the injections of fat emulsion are higher ($p<0.05$) for the LFD infants than for the AFD infants.

When the accumulation of the injected fat emulsion in plasma was related to degree of immaturity no significant relationship between the level of total lipids after the fifth injection and gestational age was found within the two groups of babies.

Lipoprotein pattern

The patterns of plasma lipoproteins before and after the injection of fat emulsion were studied and the occurrence of the pre-beta-lipoprotein fraction (pre-beta-LP) was observed (Fig 3). In the AFD group only one out of 9 infants had a trace of pre-beta-LP before the first injection as compared with all five infants in the LFD group. With the first injection of fat emulsion the LFD infants developed a marked increase in the pre-beta LP while the AFD infants showed later appearing and less pronounced pre-beta-LP. After the fifth injection of fat emulsion all infants studied demonstrated the occurrence of pre-beta-LP. The appearance of alpha lipo-proteins (alpha LP) and

No	AFD						
40	0	0	+	+	+	++	++
43	0	0	0	0	+	++	+++
44	+	++	+	+	+	+	+
50	0	+	+	++	++	+++	+++
54	0	+	++	++	++	+++	
57	0	0	0	+++	+++	+++	+++
58	0	++	++	++	++	++	+++
62	0	+	++	+	++	+	++
63	0	+		+	+	0	0
	LFD						
45	+	+	++	+++	+++	+++	+++
47	+	++	++	+++	+++	++	+++
49	+	++	++	+++	++	+++	+++
53	+	++	+++	+++	+++	+++	+++
59	+	++	++	++	++	–	–
TIME	0	40	140	240	340	440	540

Fig 3 The occurrence of plasma pre-beta-lipoproteins in the electrophoretic pattern graded from 0 to +++. No observation –

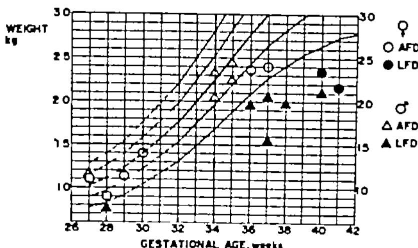


Fig 1 Relationship between gestational age and birth weight in the infants studied plotted against a Swedish reference material (4) The curves represent mean ±1 S D and ±2 S D

The effect of a single dose of heparin was also tested in the LFD babies when a significant amount of fat had accumulated

MATERIAL

All infants studied weighed less than 2 500 g at birth Only those babies were included in the study in whom the obstetrical record gave reliable data on gestational age in agreement with the findings on physical examination

The infants were separated into two major groups according to their birth weights in relation to gestational age (i) *pre-term* infants with a gestational age below 37 weeks and a birth weight above the expected birth weight minus 2 S D the AFD group and (ii) *light-for-date* infants with birth weights below the minus 2 S D line regardless of their gestational age the LFD group Eleven AFD infants and 8 LFD infants were studied Birth weights and gestational ages (g a) are shown in Fig 1 The mean values for gestational age and birth weight in the AFD group were 3 0±1 4 (S E M) weeks and 1 780±230 (S E M) g respectively In the LFD group corresponding values were 37 1±1 9 and 1 870±322

Several of the 19 babies showed mild symptoms of respiratory difficulties at the time of study No relation between development of respiratory symptoms and the injection of Intralipid® was observed in this series In 2 infants the respiratory rate even decreased markedly during the course of the study while in 2 other infants the signs of respiratory disease increased during the study One baby died 1 day after the fat injections because of severe immaturity (g.a 27 weeks)

Three of the 19 infants developed hyperbilirubinemia with maximum values at 15.3 (g.a. 34 w) 15 6 (g.a. 30 w) and 18 9 mg/100 ml (g.a 34 w) respectively at about the age of 7 days. No cases of prolonged jaundice were observed

METHODS

All infants were studied soon after birth, the studies starting between 2 and 13 hours of age The babies were nursed in incubators at their neutral temperature No food was given before or during the study Fat injections were given through an infant feeding tube (Fr 5) in either the umbilical artery (tip of catheter in aorta at diaphragm level) or umbilical vein (tip of catheter in v cava inf) The position of the catheters was controlled with fluoroscopy Between fat injections a 10% glucose–fructose solution was given at a rate of 65 ml (kg×24 hours)

Fat emulsion as Intralipid® (20% soybean oil, 1.2% lecithin and 2.5% glycerol) was given as repeated injections of 0 15 g fat/kg b w hourly over periods from 4–1 hours In 5 LFD infants a single dose of 50–100 i.e of heparin/kg b w was given after the eighth injection

To avoid fat overloading the turbidity of the plasma was measured before the next dose was given and the fat administration was interrupted when plasma showed marked turbidity (>50 NEF units).

Blood samples were drawn from the umbilical catheter after careful rinsing to avoid admixture with injected fat Blood samples of 0 8–1 0 ml were taken according to the following scheme immediately before the first fat injection and 5 20 and 40 min after this fat injection Then one sample was taken 40 min after each fat injection i e at 1 h 40 min 2 h 40 min etc In the infants who received heparin four additional samples were taken after heparin In infants with extremely low birth weight occasional samples were omitted to reduce blood loss

The following variables were studied in every blood sample total lipids according to Zöllner & Kirsch (12) lipoprotein pattern using agarose gel electrophoresis (11) and plasma turbidity using the Torpe nefelometer A visual evaluation of the lipoprotein patterns was performed without access to the patient data The quantity of prebeta-lipoprotein was estimated using a five-graded scale

RESULTS

Plasma total lipids and turbidity

The concentration of total lipids in plasma before the injection of fat emulsion was the

sum of all lipids present in plasma cholesterol cholesterol esters, triglycerides and phospholipids transported as lipoproteins and the free fatty acids (FFA) transported bound to albumin. The FFA value constitutes one portion of the total lipids The increase in total lipids after injections of fat emulsion would however mainly be due to triglycerides non hydrolysed Intralipid particles and increased pre-beta-LP Variations in alpha- and beta-LP carrying preferentially cholesterol and phospholipids and only small amounts of triglycerides would influence the total lipid value to a minor degree

Lipoprotein measurements would be a more precise way of evaluating the metabolic influence of injections of fat emulsion Lipoprotein electrophoresis allows with some accuracy the evaluation of changes preferentially in chylomicrons (and Intralipid particles) and pre-beta-LP Differences between the two groups of infants in the lipoprotein patterns during the fat elimination were observed. In LFD the elimination of exogenous fat particles was retarded and the pre beta-LP appeared already after a few hourly injections. In AFD on the other hand the pattern was characterized by the appearance of pre-beta-LP at a later time in the sequence of repeated injections and without causing any apparent influence on the elimination of the exogenous fat particles (Fig 3)

The normal catabolism of chylomicrons (10) and of Intralipid particles (9) occurs by the *hydrolysis of triglycerides with the formation* of glycerol and FFA The hydrolysis of triglycerides causes a shrinkage of chylomicrons (and Intralipid particles) an increase in particle density and the appearence of remnant particles within the density range of very low density lipoproteins (VLDL) (Fig 5) The fate of the final product 'the ghost is under discussion Animal studies utilizing labelled chylomicrons have provided evidence for an intact uptake of the remnant by the liver (2) On the other hand during chylomicron metabolism in man the hydrolysis induced by heparin injection causes an increase in high-density lipoproteins (HDL) (5) The endogenous particles secreted by the liver have the electrophoretic characteristics of pre-beta-LP and density of VLDL. The size and possibly also the electrophoretic migration rate of the remnants of chylomicrons and Intralipid particles on one hand and the endogenous hepatogenous particles on the other may coincide A possibility to verify the source of the lipoproteins occurring within the density of VLDL during the fat elimination would be by the determination of the fatty acid composition of their triglyceride moiety (studies in progress)

No relation between the ability to clear plasma from exogenous fat and the degree of immaturity was found in these groups of infants Although small the series of AFD infants contains five cases at or below 30 weeks of gestation If immaturity caused any major decrease of the ability to hydrolyse exogenous fat emulsion these infants should have demonstrated such an inability On the contrary they showed the same clearing capacity for fat as the more mature AFD infants

The hydrolysis of the lipoprotein-transported triglycerides with the formation of glycerol and FFA is dependent on the action of a lipase the lipoprotein lipase (10) Heparin induces the lipoprotein lipase reaction (10) The increased capacity for fat elimination caused by the injection of heparin therefore indicates that also in LFD infants lipoprotein lipase is available (Fig. 4)

It should be stressed that the two groups of infants studied not only demonstrated clear cut differences with regard to fat removal in between the groups but also a great variation within the groups (note the magnitude of the standard errors of mean in Figs. 2 and 4) This means that some infants in the AFD group acted more like LFD infants and vice versa The most obvious example is a pair of twins in the AFD group where the larger twin had a high fat elimination capacity while the 200 gram small twin (although above the

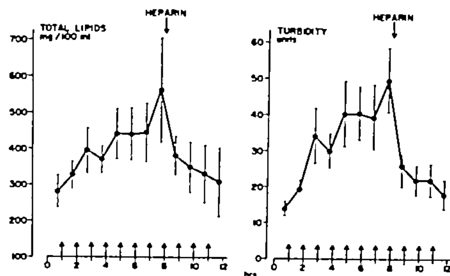


Fig 4 Concentrations of total lipids in plasma and turbidity in 5 LFD infants given heparin after the eighth fat injection Mean ±S E M are indicated Note that heparin was given as a single injection and the hourly lipid injections were continued Arrows indicate fat injections

beta-lipoproteins (beta LP) did not change in relation to injections of fat emulsion

Effects of intravenously given heparin

The injection of 50–100 IU/kg body weight of heparin (Vitrum Stockholm) to 5 LFD infants caused a marked clearing of plasma with a decrease in total lipids and turbidity (Fig 4) to a level comparable to that in the AFD infants without heparin This level in total lipids and turbidity remained low after the heparin injection in spite of subsequent injections of fat emulsion

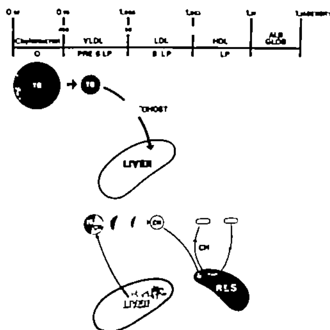


Fig 5 Schematic representation of the metabolism of Intralipid particles and plasma lipoproteins (for discussion cf ref 8)

DISCUSSION

The results from an earlier study (7) using single injections of fat emulsion to low-birth-weight (LBW) infants was confirmed by using repeated hourly injections for up to 8 hours Again in the sub-group light for-date infants (LFD) a reduced capacity for fat elimination was experienced After repeated injections of fat emulsion the most striking difference between LFD and AFD infants was observed in the turbidity of plasma the nephelometry readings (Fig 2)

Turbidity or opalescence of plasma is an expression for the presence of increased amounts of large sized lipoprotein particles >0.03 μm (6) Chylomicrons and the Intralipid particles of size 0 5–1 0 μm are the largest particles to be found in plasma Also the smaller sized pre-beta LP cause turbidity but less turbidity than their influence on the plasma total lipid values Thus the high turbidity of the plasma in LFD was mainly caused by non-hydrolysed Intralipid particles Likewise the increase of total lipids was significantly different in the two groups of infants throughout the period of repeated injections of fat emulsion (Fig 2)

The total lipid value is an expression of the

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IMPAIRED INTERFERON RESPONSE OF CHILDREN WITH CONGENITAL CYTOMEGALOVIRUS DISEASE

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ABSTRACT Emödi, G. and Just, M. (Department of Microbiology and Immunology of the University Childrens Hospital, Basel Switzerland). Impaired interferon response of children with congenital cytomegalovirus disease Acta Paediat Scand, 63: 183, 1974.—The reason for the observation, that only 1 child out of 30 newborns with congenital cytomegalovirus infection has clinical signs of congenital disease is not known. The possibility of a defect in interferon mechanism responsible for development of congenital disease was tested. Six infants with typical symptoms of congenital cytomegalovirus disease were compared with infants excreting virus without symptoms related to congenital infection and with healthy adults with and without virus excretion. From patients and controls lymphocytes were separated and stimulated by in vitro virus infection for interferon production capacity A significantly reduced interferon response was found in the children with symptoms of congenital cytomegalovirus disease, compared with the different control groups. The possibility that the impaired interferon response is a genetically fixed defect leading to generalization of cytomegalovirus infection is discussed.

KEY WORDS: Cytomegalovirus, interferon, congenital infections, immunodefects

About 1% of all newborns show excretion of cytomegalovirus (CMV) in urine after birth which often persists for many months (1 20) However only 1 child out of 30 newborns with viruria has clinical signs of congenital disease (11).

The serum antibody defense mechanism of the children with congenital CMV disease is intact The immunological response of the affected children is normal and neutralizing antibodies against CMV can be demonstrated in their sera (11)

A deficiency in interferon production might be responsible for the development of clinical symptoms after congenital CMV infection. In order to test this hypothesis we studied the interferon response of lymphocytes from patients excreting CMV in urine Circulating lymphocytes were chosen for this purpose not only because of their availability but also because of their high interferon production capacity (22) Mobile lymphocytes may be of primary importance in the interferon response to viral infection *in vivo*

MATERIAL AND METHODS

1) Six patients with symptoms of congenital CMV disease (Table 1) Patient Nr 4 was tested twice patient No 1 5 times at intervals of 1–3 weeks.
2) Ten children of the same age with CMV excretion in urine but without any symptoms related to congenital CMV infection
3) Six children of the same age without cytomegaloviruria and with no signs of infection
4) Four parents of the patients (group 1) having cytomegaloviruria but not showing any clinical signs of infection (2 tested twice)
5) Nine healthy members of the hospital staff.

Separation of lymphocytes

Heparinized blood with 5% Dextran was allowed to sedi-

minus 2 S D line) had a markedly retarded elimination

The data of the present study with repeated injections of fat emulsion confirms our earlier estimations from single injections that in AFD infants the fat elimination capacity allows the administration of intravenous fat (as Intralipid) at the hourly rate of 0 15 g/kg body weight In LFD infants on the other hand repeated injections of this amount of fat causes an accumulation of exogenous fat and remnant particles When heparin is administered simultanously with the fat emulsion the elimination capacity is restored in the LFD babies

ACKNOWLEDGMENTS

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Table 1 *Clinical virological and serological data of 6 children with congenital cytomegalovirus infection*

Case no	Age (months)	Sex	Symptoms	Virological findings		CMV specific antibodies	
				CMV in urine	in lymphocytes	IgM	IgG
1	5	M	Hepatomegaly Hydrocephalus internus	pos.	neg.	<1 16	1 1024
2	6	F	Hepatomegaly psychomotoric retardation, pathological EEG	pos.	neg.	<1 16	1 512
3	1	M	Icterus hepatosplenomegaly thrombopenia, spastic paralysis	pos.	neg.	1 256	1 1024
4	6	M	Hepatosplenomegaly recurrent respiratory infections, psychomot. retardation	pos.	neg.	<1 16	1 1024
5	2	M	Prematurity interstitial pneumonia, hepatomegaly	pos.	neg.	1 256	1 512
6	3	F	Prematurity icterus spastic paralysis, hepatomegaly seizures	pos.	neg.	<1 16	1 512

As shown in Fig 1 the same reduced interferon response of lymphocytes of patients was not only found after induction with NDV but also after induction with Sindbis virus

DISCUSSION

The diagnosis of congenital CMV disease in our patients was made by clinical symptoms and by the detection of virus in urine and the presence of CMV-antibodies (Table 1) In four of our six patients only CMV IgG and no IgM-antibodies were found The long interval between infection and antibody-testing may be an explanation for the missing IgM-antibodies Another possibility is the blocking effect of high IgG-antibody-concentrations as shown by Schmitz et al (18)

Various authors have studied the interferon production capacity of white blood cells of healthy adults (3 14). The values found in our control groups are comparable to those observed in other laboratories. According to the results of Cantell et al (3) the ability of leucocytes to produce interferon is already established in 8-week-old human foetuses No significant difference between the interferon response of leucocytes of newborns and of adults can be found

An impaired interferon production capacity was detected in patients with lymphatic leukemia (8 21) with polyglobulie (9) and uremia (16) In our patients there were no signs for such diseases In patients treated with cytostatics a diminished interferon response would not be surprising because it has been shown that such drugs (15) or antilymphocytic sera (12) are capable of inhibiting interferon production However the patients and controls in this study were not under medication.

In chronically infected tissue cultures—especially with oncogenic viruses (2)—less interferon is inductable than in virus-free control cultures.

Because a direct interaction between NDV and CMV is known (19) the possibility o

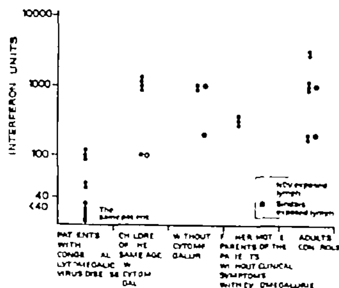


Fig 1 Interferon response of lymphocytes

ment for 1 hour at 37°C The buffy coat was harvested and washed three times in phosphate-buffered saline (PBS) Granulocytes were removed with iron powder using a strong magnet

The lymphocytes were counted and diluted in growth medium (Eagle s minimal essential medium with penicillin and streptomycin and 10% fetal calf serum) to a concentration of 8×10⁶ lymphocytes per ml Viability measurement of the lymphocytes were made at the beginning and end of the incubation period by staining with trypan blue (0 5% in saline) In no case more than 5% of non viable cells were found

Interferon induction

Two different virus strains were used for interferon induction Newcastle disease virus (California strain grown in chicken allantoic fluid) and Sindbis virus (Egypt AR 399 strain grown in human fibroblast monolayer)

10–30 HA units of Newcastle disease virus (NDV) or 10⁶–10⁶ TCD 50 units of Sindbis virus were added to 1 0 ml of lymphocytes suspension

After incubation for 24 hours at 37° in a CO_2-incubator the cells were removed by centrifugation The supernatant was adjusted to pH 2 by addition 1 M HCl and maintained at this pH for 5 days at 4 to inactivate residual virus. After readjusting the pH to 7 2 the interferon solution was stored at −20°

Interferon titration

The titration of interferon was carried out in human fibroblast cultures (WI 38) challenged with Sendai virus (grown in chicken allantoic fluid)

Difference in hemadsorption between interferon treated and non-treated cell cultures were measured by using ⁵¹Cr labelled erythrocytes (5) The amount of adsorbed erythrocytes in tissue cultures infected with Sendai virus and not pretreated with interferon is regarded as 100% adsorption. The dilution of our material resulting in a 50% adsorption-inhibition is expressed as 1 unit of interferon/ml

One of these interferon-units is equal to 1 Mill Hill unit Research Standard B 69/19 (Division of biological Standards National Institute for Medical Research, Mill Hill April 1972)

Interferon response of lymphocytes from healthy adults from the laboratory staff were included as controls in each tests of interferon induction and interferon titration

Tests for determination of free interferon in serum were done as mentioned above

Virus studies

Isolation of CMV from urine was achieved by inoculating 5–6 tubes of confluent cultures of WI 38 human fibroblast cells (Eagle s minimal essential medium containing 2% inactivated calf serum) with samples of freshly voided clean urine For isolations of CMV from lymphocytes samples of the same lymphocyte suspensions were used as for interferon induction studies. Tubes with cytopathogenic effect (CPE) were subcultured and the CMV was identified by the following criteria. specific CPE in WI 38 cell cultures presence of intranuclear inclusion bodies. lack of CPE in monkey kidney and mouse L cell cultures

Antibody studies

Complement fixation (CF) tests were done by a micromethod (10) Cytomegalovirus-immunofluorescence-IgM and IgG were measured by an indirect method (17).

RESULTS

The detailed results of the interferon response from the lymphocytes of the patients and the controls can be seen in Fig 1 The interferon response of the patients with the symptoms of congenital CMV disease are significantly below the level of the various control groups

One of the patients was tested 5 times at 1-3-week intervals during a period of 10 weeks. As seen in the table the five results from the same patient differ only slightly and are always less than 40 units of interferon/ml

In children without any clinical signs of infection there is no difference in the interferon response regardless as to whether they show cytomegalovirus excretion (group 2) or not (group 3) No difference in interferon production by lymphocytes was found between children and adults

Adults with CMV excretion in urine (group 4 parents of our patients group 1) have the same interferon response as adults without virus excretion (group 5)

All lymphocyte cultures—also these of our patients of group 1—were free of CMV No free interferon was detected in the sera of CMV-infected patients

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impaired interferon induction by NDV in the lymphocytes of CMV patients due to the presence in the white blood cells (13) must be considered But from the patients with CMV disease no CMV were detected in the lymphocytes during the period of the interferon study Moreover a similar impairment in the interferon response of our patients was also observed after interferon induction with Sindbis virus

Measles vaccinations can be used for *in vivo* induction of human interferon Three children with cytomegalovirus infection disease were vaccinated against measles by Glasgow et al (7) Two of the vaccinated showed an *in vivo* interferon response Our results are not in disagreement with the *in vivo* tests of Glasgow because although the interferon responses of the lymphocytes of our patients were significantly reduced they were not abolished (We intend to vaccinate our patients against measles after 1 year of age and compare the interferon response after vaccination with the response of healthy children from the control group)

Two general possibilities regarding the impaired interferon response of the lymphocytes of our patients must be discussed—is the diminished interferon production a primary or a secondary effect? It is possible that in the first weeks or months of pregnancy foetal lymphocytes are damaged by a massive viral infection resulting in an impaired function concerning the ability for interferon production The impaired defense mechanism could lead to generalization of the CMV infection

To our knowledge it is not known whether the interferon response is also diminished after other embryonic or foetal infections—for instance rubella In a paper published by Desmyter et al (4) 11–18-month-old children with congenital rubella syndrome vaccinated against measles showed an interferon response similar to that of normal children The interferon responses of leucocytes were not examined Because it is known that at CMV is rather resistant to the action of interferon and therefore high interferon concentrations may be needed for preventing virus dissemination CMV infection might be a higher risk than rubella

The possibility that lymphocytes of our patients are rendered hyporesponsive to the in vitro infection (NDV or Sindbis virus) by interferon induced *in vivo* by the CMV infection is unlikely because in the same blood samples in which separation of lymphocytes for interferon stimulation were done no circulating interferon in serum was present

It is not possible to exclude a primary defect in interferon host defence mechanism in our patients showing the impaired interferon response If impaired interferon production capacity would be the primary reason for dissemination of the CMV infection a genetically fixed defect would be possible Since CMV infection occurs in 1% and CMV disease in 0 03% of newborns such a genetic impairment of the interferon defense mechanism would be a frequent defect Studies concerning the possibility of a relationship between cellular immunity and interferon response are in progress (6)

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FLUORIDE MINERALISATION DEFECTS OF THE ENAMEL AND TOOTH WIDTH

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ABSTRACT Grahnén, H., Lysell L., Myrberg N and Ollinen, P (Departments of Pedodontics and Orthodontics, University of Umeå Sweden and Department of Orthodontics, University of Amsterdam The Netherlands) Fluoride Mineralisation defects of the enamel and tooth width Acta Paediat Scand 63 188 1974.—The purpose of the investigation was to appraise the frequency of enamel mineralisation defects in 151 children aged 8–11 years, born and raised in a district in Västerbotten county in northern Sweden, where the fluoride content of the drinking water ranges up to 2 mg/l and to examine the mesio-distal widths of the teeth for effects of fluoride in these concentrations. The control group consisted of 213 children who had always lived in a town where the fluoride content of the drinking water was ≤0 1 mg/l The mineralisation defects were recorded clinically and photographically Owing to the various ages of the children, only mineralisation defects in permanent incisors and six year molars were noted. The mesio-distal widths of the teeth were measured on plaster casts. The frequency of enamel fluorosis was high (34%) in those children whose drinking water had a fluoride content of 1 0–2 0 mg/l and also high (31%) in those children living in areas with a fluoride content of 1 0–1.2 mg/l. No satisfactory explanation can be offered for the relatively high frequency of fluorosis. There are however variations in the natural fluoride concentration of the ground water and probably also considerable individual variation in water consumption. This stresses the necessity of further investigation of children s fluoride intake No significant differences in mesio-distal widths of individual teeth could be demonstrated

KEY WORDS Fluorine intake dental fluorosis, enamel hypoplasia tooth morphology (tooth width)

According to the literature the optimal concentration of fluoride in drinking water in the prophylaxis of caries and one which does not produce cosmetically disturbing enamel fluorosis is about 1 mg/l (e g 1 2 4 6 14 15 19 20 22) This has also been found in Swedish investigations (9 16 21 23)

Whether fluoride in the drinking water has any effect on the width of teeth is still debatable In rat a decrease has been reported (17) in man an increase (26) a reduction (7) or no change (3)

The purpose of the present investigation was to find out whether children born and raised in a district where the fluoride content of the drinking water ranges up to 2 mg/l differed from populations using unfluoridated water regarding the frequency of enamel mineralisation defects and the mesiodistal width of the teeth

MATERIAL

The original material consisted of all the 182 six to nine-year-old children living in the neighbouring villages Ersmark Kusmark and Kåge in 1966 This district is situated near the coast of the Gulf of Bothnia in the County of Vasterbotten in northern Sweden. All the children were examined except 4 who were ill at the time of the investigation. In 24 cases the fluoride content of the drinking water during the periods of mineralisation of the teeth could not be determined because the families

Table 1 *Water works in the district*

Place/water works	Start year	Depth of well (m)	*Fluoride content (mg/l)*				
			1964 Feb	1965 Sept.	1966 April	1966 Sept.	
Ersmark/Well I	1957	50		1 0	1.2		Interconnected
Well II	1950	70					
Kusmark/Well I	1940	83		1.0	1 0		Interconnected
Well II	1964	75					
Kåge/Well I	1942	70	0 9	1 0	1 4	1 0	
Well II	1940	80		0 8	0 6	0.7	

were relative newcomers to the district. The remaining material thus consisted of 154 children

As control material 230 children aged 6–9 years were chosen at random, 225 cooperated. All of them had grown up in the town of Skellefteå, situated close to the three villages, where the fluoride content of the drinking water was ≤0 1 mg/l None of the children had spent their summer holidays in districts where the drinking water had a higher fluoride content.

151 children (78 boys and 73 girls) in the test group and 213 (104 boys and 109 girls) of the controls were re-examined in 1969–70.

METHOD

All of the children were examined clinically and roentgenologically at a dental clinic The teeth were examined for mineralisation defects of the enamel under dental operating light and with fibreglass optic All of the teeth were examined but owing to the variation of the children s ages and number of erupted permanent teeth only observations made on the permanent incisors and 6-year molars are accounted for here. Fluorosis was diagnosed and graded according to Dean (8) and Møller (19) other defects of enamel mineralisation, according to Zimmerman (27) and Grahnén & Selander (17) To permit comparison with clinical observations, colour photographs were taken of the dentition at the first examination and of all cases with defects of mineralisation demonstrated at the second examination (camera=Nicon Medical film=Kodak Ektachrome X) Good agreement was found between the mineralisation defects observed on the two occasions The photographic findings also agreed well with the clinical findings.

Notes were made of the following points in the history place of birth and childhood and place where summer holidays were spent, drinking water general state of health and mode of feeding in infancy and supplementary fluoride, as in tablets, dentifrice or tea

The widths of the teeth were measured with sliding caliper according to Lundström (18) on casts made from the alginate impressions obtained at the first examination Measurements were made of the mesio-distal widths of permanent teeth, from the first molar on the left side to the corresponding tooth on the other side and of all primary teeth with the exception of the central incisors

In 1964–66 the fluoride content of the water in the districts in question was determined on various occasions in order to detect variation with the season of the year or with the level of the groundwater (Table 1). These determinations were performed at the water laboratory Apoteket Järven, Umeå using a colorimetric method (method No. 1 for fluoride according to the recommendations No 122 of the Medical Board 1967 concerning physicochemical examination of the water)

However not all of the households were supplied by the public water works. The fluoride content was therefore also determined in water from 49 private water supplies in these three districts. As expected, the fluoride content varied with the depth of the ground water The highest fluoride content was 2.0 mg/l.

In the statistical treatment Student s *t*-test and χ^2 analysis were used (24).

RESULTS

Since none of the parameters studied concerning enamel defects was found to vary with sex the results of this analysis are given for both sexes taken together

The mineralisation defects of enamel found in the test group and in the controls are given in Table 2 It is clear from the table that in those children whose drinking water had a fluoride content of ≤0.5 mg/l the frequency of defects (Total fluorosis Total not fluorosis Grand total) was of the same order as in the control group The table also shows that at a fluoride content of ≥1 0 mg/l the frequency of enamel fluorosis was markedly increased Fluorosis was thus seen in 3% where the fluoride content of the drinking water was ≤0 5

Table 2 *Occurrence of enamel mineralisation defects in permanent incisors and six year molars*

No of children	F-content (mg/l)	Number of children with fluorosis graded according to Dean				Total fluorosis (%)	Number of children with enamel mineralisation defects not fluorosis		Total not fluorosis (%)	Grand total (%)
		0 5	1	2	3		Hypoplasia	Opacities		
Test group										
54	<0.5		1			2	1	12	24	26
24	0 6–0 9	1				4	1	10	46	50
67	1 0–1 2	4	8	7	2	31	3	15	77	98
6	1 3–2 0	1	1	1	1	67				67
78	<0 9	1	1			3	2	22	31	33
73	1 0	5	9	8	3	34	3	15	25	59
Control group										
213	<0 1	–				0 9	11	15	21	22

Deans classification

Questionable (0 5): The enamel discloses slight aberrations from the translucency of normal enamel, ranging from at few flecks to occasional white spots

Very mild (1) Small opaque paper white areas scattered irregularly over the tooth but not involving as much as approx 25% of the tooth surface

Mild (2): The white opaque areas in the enamel of the teeth are more extensive but do not involve as much as 50% of the tooth.

Moderate (3): All enamel surfaces of the teeth are affected and surfaces subject to attrition show marked wear Brown stain is frequently a disfiguring feature

mg/l and in 34% where it was ≥1 0 mg/l The difference was statistically significant ($p <$ 0 001) The frequency of fluorosis was also very high (31%) in those children whose drinking water had a fluoride content of 1 0–1 2 mg/l Comparison of the total frequency of mineralisation defects not registered as fluorosis showed no significant differences Neither were any statistically significant differences in frequency of mineralisation defects other than fluorosis demonstrable between the test group and the controls The two groups did not differ notably from one another regarding supplementary supply of fluoride or general health as judged from anamnestic data

No matter how the widths of the individual mesio-distal teeth were compared the agreement was strikingly good The expected sex differences were uniform and systematic in all of the groups Neither was any tendency to reduction or widening of the teeth demonstrable in children with enamel fluorosis

DISCUSSION

As pointed out in the introduction a fluoride content of drinking water of about 1 mg/l has been generally accepted as the optimal prophylactic concentration which does not cause cosmetically disturbing enamel fluorosis But the present investigation showed that clinically demonstrable enamel fluorosis even cosmetically disturbing (e g Dean s class 3) may develop when the fluoride concentration is not notably higher than 1 mg/l Enamel hypoplasia was also noted in three of the children with fluorosis (6-year molars) This is remarkable in the light of the fact that the occurrence of enamel fluorosis in association with a certain fluoride content should be lower in northern latitudes than in districts in warmer zones owing to the greater consumption of water in the latter The consumption of water usually varies with the mean maximum temperature (10 22)

No clear explanation can as yet be offered for the relatively high frequency of enamel

fluorosis in the material studied It may be pointed out, however that in a recent investigation (21) of the frequency of enamel fluorosis in 653 children (age 15–16) who had grown up in Uppsala Sweden where the drinking water has a fluoride content of 1 0 mg/l the frequency of enamel fluorosis in the children who had always lived in Uppsala was still higher (60% according to Dean s classification 1–3) The method of examination was comparable to ours except that we also used a fibreglass optic Ribelius & Torell (21) however did not report the frequency of enamel mineralisation defects not ascribable to enamel fluorosis. Neither did they mention any case of enamel hypoplasia and they reported that none of the opacities of the enamel were esthetically disturbing However Dean s class 3 was noted in 3%

A high manganese content of the drinking water is claimed to have a certain discolouring effect on the teeth (11 21 25) The manganese content of the water supplies (Ersmark Kusmark and Kåge) ranged from <0 05–0 20 mg/l

Bonham et al. (5) and Hamn (13) have pointed out that in the estimation of water consumption one should consider not only the mean maximum temperature but also the humidity of the atmosphere This is extremely low in northern Sweden during the coldest months of the year

As for the concentration of fluoride in other public water supplies in the district determinations in September 1965 and April 1966 showed that it varied most in the deep wells nearly all of which had a higher fluoride content in the spring, before the ground has begun to thaw than in the autumn. Differences of up to about 1 mg/l were reported

The fluoride content may also vary with the amount of water removed as is apparent from an investigation in progress in another district in the area of Västerbotten There are thus several factors capable of influencing children s fluoride intake and they may have affected the results of the present investigation which was carried out on a relatively small material But even if these disturbing factors be considered the frequency of enamel fluorosis still appears high The investigation clearly suggested that the naturally occurring fluoride content of drinking water should be regularly checked even if it is only about 1 mg/l

No systematic differences in the mesio-distal widths of single teeth were found Neither was any tendency to a widening or reduction of the teeth demonstrable in those children in whom enamel flurosis was diagnosed This investigation therefore lends no support to the optimal prophylactic concentrations in man has any effect on the width of the teeth

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No clear explanation can as yet be offered for the relatively high frequency of enamel

^{99m}TC PERTECHNETATE BRAIN SCINTIGRAPHY IN CHILDREN

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ABSTRACT Edeling, C. J. (Dept of Nuclear Medicine Rigshospitalet, Copenhagen, Denmark). ^{99m}Tc-Pertechnetate brain scintigraphy in children. Acta Paediat Scand, 63:193, 1974.—One hundred and sixteen children all suspected of brain disease were examined by ^{99m}Tc-pertechnetate scintigraphy Twenty-fiv of 34 verified intracranial tumours or tumourlike diseases accumulated ^{99m}Tc Intracranial lesions such as leukaemic infiltrations, subdural haemorrhage, brain abscess, and subdural abscess were also detected by this method. Three case histories are reported: astrocytoma in the cerebellum, tuberous sclerosis, and brain abscesses. The choice of isotopes for brain scintigraphy and the advantages and disadvantages of the method are discussed. It is concluded that ^{99m}Tc-scintigraphy seems to improv the accuracy in diagnosing brain neoplasms in children.

KEY WORDS: Brain scintigraphy neoplasms of the nervous system, tuberous sclerosis, brain abscess, leukaemia

cintigraphy for the visualizing of focal intra-ranial lesions has become a regular diagnostic rocedure The technique offers several advan-tges, compared with cerebral angiography or neumoencephalography

Brain scintigraphy in adults has been exten-ively reviewed (4 6 10 11 13) Reports bout its use in children have mostly been estricted to limited numbers of cases (? 3 5 8, 9 12)

Results are presented of ^{99m}Tc-pertechne ate brain scintigraphies carried out on chil lren during a 6-year period (1966–71) in our lepartment

MATERIAL AND METHODS

One hundred and sixteen children of both sexes, ranging n age from 39 days to 15 years, were examined by scintigraphy The children wer all suspected of having brain disease

The scintigraphic procedure was initiated by giving potassium perchlorate (200 mg) orally to the patients before administration of the radionuclide Atropine was not used. Premedication with sedatives was necessary in most cases of the small children in order to avoid any movement during the scanning procedure Some –14 mCi ^{99m}Tc as sodium pertechnetate was injected intravenously In four cases, the dose was given orally The universal radiation dose to a child has been calculated to less than 0 2 rad per mCi of ^{99m}Tc (?) The scintigraphies were performed 5–15 minutes after the injection and 30 minutes after an oral dose No late studies were performed The children were examined by means of a rectilinear scanner (1966–69 Nukab 3× inch Scanner 1969–71 Picker 5×? inch Nuclear Magna Scanner 500 both provided with a multi-hole focusing collimator).

Usually the examination started with a lateral scintigram from the side suspected of focal lesion Subsequently the appropriate anterior or posterior scintigram was made The time necessary to obtain a scintigram was approximately 30 minutes The results were stored on magnetic tape (Nukab Memonukleograph), and for each of the studies series of reproductions was made with different subtraction levels.

For scintigram to be considered positive" an increased accumulation ^{99m}Tc had to be detected on the lateral scintigram as well as on the corresponding anterior or posterior scintigram I case of a noticeably increased accumulation in one projection only the scintigram was interpreted as suspect of a pathological accumulation.

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Table 3 *Results of scintigraphy in 80 patients with diagnoses based on clinical course electroencephalography and other clinical investigations*

Diagnosis	No	^{99m}Tc-scintigraphy +	^{99m}Tc-scintigraphy −	Arteriography +	Arteriography −	Arteriography 0	Pneumoencephalography +	Pneumoencephalography −	Pneumoencephalography 0	Electroencephalography +	Electroencephalography −	Electroencephalography 0	Other investigations
Hydrocephalus	4		4		2	2	4			2	2		
Encephalopathy	7	1	6	1	2	4	4	3		7			
Optic neuritis	1		1		1			1		1			Ophthalmoscopy
Encephalitis	1	5+1	6	1	9	2	3	9		8	4		In 4 cases histologic examination
Meningitis	4		4			4	1		3	1	3		
Abscess, brain	5	5		5			3		2	5			Surgery
Abscess, subdural	2	1	1	1		1	1		1	1			Surgery
Myxedema	1		1			1	1			1			^{131}I-thyroid test
Bone defect	1		1		1				1			1	X-ray examination
Subdural haematoma	1	1		1			1			1			Surgery
Cerebral embolism obs. Mb. Cordis	1		1			1			1	1			
Essential hypertension Mb. Cordis	1		1		1			1		1			
Epilepsy	27		27	2	16	9	9	14	4	25	2		
Migraine			2		1	1		1	1	1	1		
Leukaemia	3	2	1			3	1		2	2	1		2 pts. had lymphoblasts in CBS
Lymphosarcoma	1		1			1	1			1			
Neuroblastoma, retroperitoneale	2		2			2		1	1		2		Surgery
Drusen in the eye	1		1			1		1		1			Ophthalmoscopy
Dystrophia adiposo genitalis	1		1		1			1	1				
Unclarified	3	1*	2		2	1	2	1		1	2		
Total No	80												

* Positive scintigraphy due to burr-holes.
+ = positive − = negative, 0 = no study performed.

scintigraphy Histological examinations of these three lesions showed no signs of malignancy

One case of cephalic neurocutaneous haemangiomatosis and one tumour interpreted as benign *were not histologically verified. Infra*tentorial neoplasms were recognized in 6 of 8 cases. Two medulloblastomas located in the posterior fossa were not visualized by scintigraphy None of the four brain stem tumours were positive in the scintigrams Three brain stem tumours and one tumour located infratentorially were not verified histologically but were interpreted as malignant and the patients were in all cases given x-ray treatment afterwards.

Arteriography was performed in 23 cases and in 21 of these the agreement with the diagnosis was good (91%) Pneumoencephalography was undertaken in 30 cases 24 of these showed good correspondence with the diagnoses (80%)

For further details see Table 2

Table 3 shows the results of scintigraphy compared to arteriography pneumoencephalography and electroencephalography in 80 patients with diagnoses based on the clinical course encephalography and other clinical investigations Definite diagnoses verified by histological examination or pneumoencephalography were obtained in 17 cases of diseases which were not primary neoplasms of the nervous system They were two cases of leukaemic infiltration (2 positive scintigrams 1 positive PEG) 1 case of subdural haemorrhage (positive scintigram arteriography

Table 1 *Results of ^{99m}Tc scintigraphy compared with diagnoses in 116 patients*

Result of scintigraphy	Pathological cases A	Pathological cases B	Normal cases
Positive	25	17	0
Negative	9	63	2

A = patients with intracranial neoplasms of the nervous system histologically or otherwise verified
B = patients with diagnoses based on the clinical course electroencephalography or other clinical studies

RESULTS

Table 1 shows the results of ^{99m}Tc scintigraphy compared with diagnoses in 116 patients 42 scintigrams were positive 25 from a group of patients with confirmed neoplasms of the nervous system 17 from a group of patients with diagnoses based on the clinical course encephalography and other clinical investigations 74 scintigrams were negative Two of these were obtained from normal patients

Table 2 indicates the results of scintigraphy pneumoencephalography and electroencephalography in 34 patients with intracranial neoplasms of the nervous system 25 of 34 verified neoplasms were detected by scintigraphy (74%)

Supratentorial neoplasms were localized in 19 cases Two cases of malformation of cerebral vascular and perivascular structures and one case of dermoid cyst were negative by

Table 2 *Result of scintigraphy compared to arteriography pneumoencephalography and electroencephalography in 34 patients with intracranial tumours and tumour like diseases*

Site and type of lesion	^{99m}Tc-pertechnetate scintigraphy pos	neg.	Arteriography pos	neg.	Pneumoencephalography pos	neg.	Electroencephalography pos	neg.	Histological verification
Supratentorial 22									
Astrocytoma	7	0	7	0	5	1	6	1	+
Astroblastoma	1	0	1	0	–	–	1	0	+
Glioblastoma multiforma	1	0	1	0	–	–	1	0	+
Ependymoblastoma	2	0	2	0	1	–	2	0	+
Medulloblastoma	1	0	1	0	–	–	1	–	+
Sympathicoblastoma	1	0	1	0	1	0	1	0	+
Neurinoma (v Recklinghausen	1	0	1	0	1	0	1	0	+
Malform. of cerebr vascular and perivascular structures		2	4	0	3	1	4	0	+
Cephalic neurocutaneous haemangiomatosis	1	0	0	1	0	1	1	0	–
Tuberous sclerosis	1	0	0	1	0	1	1	0	+
Tumour benign	1	0	1	0	1	0	1	0	–
Dermoid cyst	0	1	–	–	1	0	1	0	+
Infratentorial 8									
Astrocytoma	2	0	–	–	2	0	2	0	+
Ependymoma	1	0	–	–	1	0	1	0	+
Medulloblastoma	2	2	–	–	4	0	4	0	+
Tumour not classified	1	0	1	0	1	0	1	0	–
Brain stem 4									
Astrocytoma	0	1	–	–	0	1	1	0	+
Tumour not classified	0	3*	1	–	3	1	3	–	–
Total 34	25	9	21		4	6	33	1	

Tumour verified by pneumoencephalography



Fig. 2

frontal lobe. At surgery a large irregular tumour was found in the right frontal lobe.

Final diagnosis: Tuberous sclerosis in the right frontal lobe.

Case 3

Clinical history: A 7 year-old boy with congenital heart failure. One week prior to admission he developed a persistent fever. On admission he was comatose. Bilateral papilledema was stated. EEG showed no focal abnormality.

Preliminary diagnosis: Meningitis.

Scintigraphs: The right lateral view showed an area of abnormal activity adjacent to the base of the skull in the frontal region. In the left lateral view (*a*) there is abnormal activity in the parietal region and in the frontal region close to the skull. In the anterior view (*b*), there is abnormal activity in both hemispheres. In all views the abnormal accumulations have an area of decreased activity in the centre (halo effect).

Subsequent course: A right carotid angiogram revealed medial displacement of the anterior artery. Left carotid angiogram showed downward displacement of the pericallosa artery on a level with the coronal suture. Craniotomy was carried out, and abscess cavities were aspirated and removed. Cultivation showed growth of streptococci.

Final diagnosis: Brain abscesses.

DISCUSSION

The pathology and location of an intracranial lesion may influence its detectability by scintigraphy

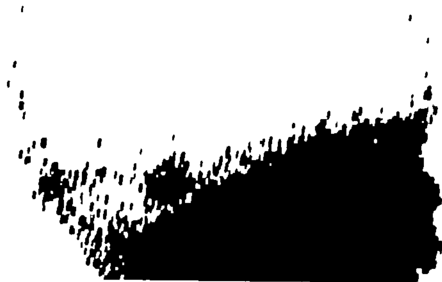


Fig 1

PEG and EEG) 4 cases of encephalitis 5 brain abscesses (agreement with arteriography 100 %) 1 subdural abscess and 4 hydrocephalus (scintigrams all negative PEG all positive)

scintigraphy with ^{203}Hg-chlormerodrine ^{197}Hg-chlormerodrine and ^{131}I HSA Seven in 2 patients with encephalitis (one of them had burr-holes) and in one case of encephalopathy In the remaining 63 cases the scintigrams were all negative

CASE REPORTS

The following cases have been selected to demonstrate

1) Visualization of tumours in the fossa posterior by scintigraphy (in the present material 6 of 8 were positive on the scintigram but normally this is a difficult area)

2) One case of rare disease of the nervous system which was positive by scintigraphy

3) The importance of scintigraphy for localizing one or several brain abscesses in order that the surgeon may discern them clearly before attempting evacuation

Case 1

Clinical history A ? year-old girl who started hav fits of screaming ? months prior to admission Op thalmoscopy indicated bilateral papilledema.

Preliminary diagnosis Posterior fossa tumour

Scintigraphy In the right lateral view abnorm activity in close relation to the anterior part of t tentorium cerebelli (Fig. 1) Posterior view normal.

Subsequent course Pneumoencephalography ind cated a tumour in the cerebellum At surgery a cyst astrocytoma was found in the anterior part of the rig cerebellar hemisphere

Final diagnosis Grade II astrocytoma in the rig cerebellar hemisphere

Case 2

Clinical history A 9-year-old girl with adenoma seb ceum in the face Since 3 months old she had had ep leptic attacks and mental retardation 2 months pri to admission a decrease in vision was noticed On ad mission the patient was blind Ophthalmoscopy ind cated bilateral papilledema. Other neurological signs wer spasticity of the left limbs X-ray of the skull suggeste increased intracranial pressure

Preliminary diagnosis Brain tumour

Scintigraphy In the anterior view (*a*) there is a area of abnormal activity at the base to the right o the midline The lesion is seen in the fronto-tempora region on the right lateral scan (*b*)

Subsequent course A right carotid angiogra revealed displacement of the anterior arteries and dow ward displacement of the lenticulostriated arteries. Pne moencephalography indicated a tumour at the base in th

pediatric neurological and neurosurgical departments who were suspected of focal intracranial lesions Six of 9 neoplasms were positive by scintigraphy (35%). The radiopharmaceutical used were ^{131}I HSA ^{203}Hg-chlormerodrine and ^{197}Hg-chlormerodrine Lorentz et al (8) examined 47 children with tentative diagnoses of brain tumour Eleven of 17 neoplasms were detected (65%) The radiopharmaceuticals used were ^{203}Hg-chlormerodrine and ^{197}Hg-chlormerodrine Lincke (7) carried out brain scintigraphy in 71 children using ^{197}Hg-chlormerodrine It was not specified whether the study was carried out on selected patients Sixten of 35 neoplasms were detected (46%)

Koos et al (5) used ^{99m}Tc-pertechnetate 182 children suspected of intracranial lesions were examined. Thirty-nine of 43 neoplasms were detected (91%) In 31 cases the tumour was verified by histological examination Twenty-nine of these showed a positive scintigram (93%) One medulloblastoma and one astrocytoma in the posterior fossa were not recognized by scintigraphy In 12 cases, the tumours were not classified but verified by one or several neuroradiological methods Ten of these were positive by scintigraphy (83%) and two located in the central part of the brain, were negative. The diagnoses of the remaining 139 children are not mentioned in the study

Frigeni et al (3) examined 105 children by brain scintigraphy One group of these patients was given ^{203}Hg-chlormerodrine another group received ^{99m}Tc-pertechnetate In the first group 27 of 30 neoplasms were detected (90%) In the other group 9 of 10 neoplasms (90%) were positive In 21 of 40 tumours the diagnoses were verified histologically in 19 cases by pneumatic studies

From the various reports it appears that brain scintigraphy is very useful for detection and localization of brain neoplasms in children.

In the present study and in those of Mealey (9) David et al (2) Lorentz et al (8) and Lincke (7) no positive scintigrams were obtained in children with neoplasms of the brain stem Koos et al (5) reported positive scintigrams in three children with tumours of the brain stem (no histological examination) and Frigeni et al (3) reported eight positive scintigrams in 9 children with brain stem tumours (no histological examination) In the present material a case of neurinoma von Recklinghausen one cephalic neurocutaneous haemangiomatosis and one rare case of tuberous sclerosis showed positive scintigrams Leukaemic infiltrations were also recognized as in the work of David et al (2) Brain abscesses were detected in 5 of 5 cases (David et al (2) had 2 of 2 cases, Lorentz et al (8) 4 of 4)

^{99m}Tc-scintigraphy seems to Improve the accuracy in diagnosing brain neoplasms The present study shows an accuracy of 74% Koos et al (5) 91% Frigeni et al (3) 90% In other studies with other radiopharmaceuticals the accuracy was. Tefft et al. (12) 57% Mealey (9) 42% David et al (2) 35% Lorentz et al (8) 65% and Lincke (7) 46% ^{99m}Tc-pertechnetate gives the smallest universal radiation doses per 100 μCi as compared with ^{131}I HSA ^{203}Hg-chlormerodrine and ^{197}Hg-chlormerodrine Furthermore ^{99m}Tc-pertechnetate gives no special critical organ doses if the thyroid is blocked with potassium perchlorate (2)

Concerning the mechanism of accumulation of ^{99m}Tc-sodium pertechnetate, Baum (1) investigated brain tumours by means of autoradiography In a mouse glioma, the radioactivity was present only in tumour cells whereas there was no evidence of activity in the small blood vessels or in the intercellular spaces He reported that initial human studies have shown a similar accumulation in brain tumour cells

The disadvantages of brain scintigraphy with ^{99m}Tc-pertechnetate are that the scanning procedure is rather time consuming and not yet available to all pediatric departments The use of scintillation cameras may speed up the procedure considerably

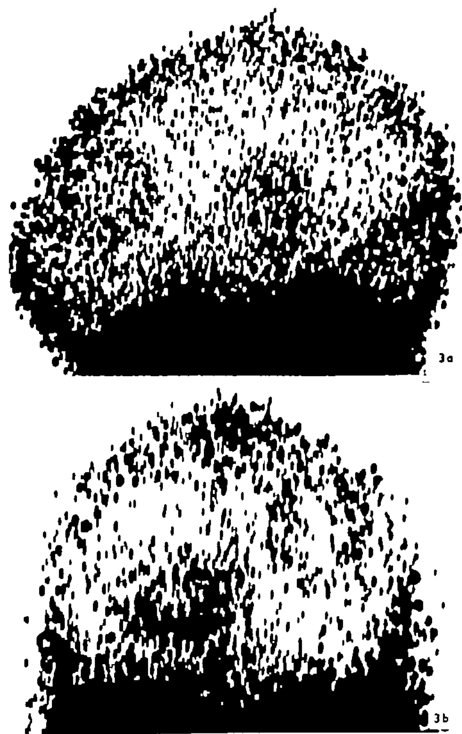


Fig 3

Concerning intracranial neoplasms of the nervous system 25 of 34 were detected by scintigraphy (74%) in this study Four of four brain stem tumours and two medulloblastomas located infratentorially were not recognized Three non-malignant lesions located supratentorially were not detected The scintigraphic investigations in this study correspond to the results of others Tefft et al (12) used ^{197}Hg-chlormerodrine for scintigraphy of 86 children Thirteen of 23 neoplasms of the brain were positive in the scintigrams (57%). Mealey (9) examined 50 children by brain scintigraphy with ^{203}Hg chlormerodrine ^{197}Hg-chlormerodrine and ^{131} HSA Seven of 17 neoplasms were positive on the scintigrams (42%) David et al (2) carried out scintigraphy of the brain in 220 children from

pediatric neurological and neurosurgical departments who were suspected of focal intracranial lesions Six of 9 neoplasms were positive by scintigraphy (35%). The radiopharmaceutical used were ^{131}I HSA ^{203}Hg-chlormerodrine and ^{197}Hg-chlormerodrine Lorentz et al (8) examined 47 children with tentative diagnoses of brain tumour Eleven of 17 neoplasms were detected (65%) The radiopharmaceuticals used were ^{203}Hg-chlormerodrine and ^{197}Hg-chlormerodrine Lincke (7) carried out brain scintigraphy in 71 children using ^{197}Hg-chlormerodrine It was not specified whether the study was carried out on selected patients Sixteen of 35 neoplasms were detected (46%).

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The disadvantages of brain scintigraphy with ^{99m}Tc-pertechnetate are that the scanning procedure is rather time consuming and not yet available to all pediatric departments The use of scintillation cameras may speed up the procedure considerably

A positive scintigraphy does not differentiate between malignant and benign lesions but it had no difference upon the treatment of the patients because benign as well as malignant brain neoplasms require surgery Furthermore the anatomical relationship of the lesion to blood vessels and the ventricular system is not demonstrated by scintigraphy Finally a negative scintigram does not rule out a possible neoplasm this calls for additional electroencephalography and other extensive studies

Brain scintigraphy offers several advantages It is relatively easy to perform as compared to angiography and to pneumatic studies it is adaptable for use on outpatients and it appears to present minimal risk Furthermore brain scintigraphy does not alter the intracranial pressure dynamics It can therefore be used with safety even in cases of increased intracranial pressure Finally it can give satisfactory information concerning the location and sometimes also the size of intracranial lesions whereas angiography or PEG can only give an idea of these factors from the degree of distortion or displacement of the surrounding anatomical points

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ESTIMATION OF FREE THYROXINE INDEX IN THE NEWBORN USING MICRO-METHODS

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ABSTRACT Rogowski, P., Siersbæk-Nielsen, K. and Mølholm Hansen, J. (Medical Department E, the Obstetrical Department, and the Department of Clinical Chemistry Frederiksberg Hospital, Copenhagen, Denmark) Estimation of free thyroxine index in the newborn using micro-methods. Acta Paediat Scand, 63:201 1974.—Thyroid function in the newborn has been studied with the purpose of establishing normal values for total plasma thyroxine, T 3 test and free thyroxine index in the neonatal period using new micro-methods. Total thyroxine determinations were carried out using the Sephadex column method (Tetralute®) which requires 25 to 50 μl plasma. The unbound TBG binding sites were evaluated using T 3 Sephadex retention test (Trilute®) requiring 50 μl plasma. Free thyroxine index were calculated as the product of the two tests. 202 full-term newborns were examined in the period 19 to 71 hours after birth and the normal range (95 % limits) for plasma thyroxine were found to be 9.2–26.0 μg/100 ml. Normal values for the T 3 test varied between 42.5 and 64.9 % and free thyroxine index values between 510–1378 arbitrary units. The mean values of total thyroxine, T 3 test and free thyroxine index were found to be significantly increased compared with cord blood and adult mean values indicating physiological thyroid hyperfunction in the neonatal period. The new thyroid function tests used in the present study were found to be technical simple and are suggested to be used whenever thyroid diseases in the newborn are suspected.

KEY WORDS: Neonatal, thyroid function, micro-methods

Several studies of the thyroid function in the newborn have demonstrated a physiological hyperfunction of the thyroid gland (4 5 6 7 12, 13 14 15 18)

Serum protein-bound iodine butanol-extractable thyroxine ^{131}I-triiodothyronine uptake in erythrocytes or resin (T_3-tests) dialysable thyroxine and ^{131}I uptake in the thyroid gland have been found to be increased in the first days after birth. The increase in the parameters of thyroid function follows an acute release of thyrotropin (TSH) in the first hours of life (20)

In a previous study we developed micro-thyroid function tests for a longitudinal study of neonatal thyroid function in a smaller group of infants (15) Recently technically very simple and commercially available micro-methods for the measurement of thyroxine (Tetralute®) and T_3-test (Trilute®) have been introduced (1 2 8 16 17)

The purpose of this study has been to examine a larger group of children (with the use of Tetralute® and Trilute® tests) to establish normal values for total thyroxine T_3-test and free thyroxine index in the neonatal period.

A positive scintigraphy does not differentiate between malignant and benign lesions but it had no difference upon the treatment of the patients because benign as well as malignant brain neoplasms require surgery. Furthermore the anatomical relationship of the lesion to blood vessels and the ventricular system is not demonstrated by scintigraphy. Finally a negative scintigram does not rule out a possible neoplasm this calls for additional electroencephalography and other extensive studies.

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The purpose of this study has been to examine a larger group of children (with the use of Tetralute® and Trilute® tests) to establish normal values for total thyroxine T_3-test and free thyroxine index in the neonatal period

Table I *Mean values SD and range from 202 newborn full term infants and 68 normal adult females*

	Full-term infants		Normal adults	
	Mean±S D	Range	Mean±S D	Range
Serum thyroxine (μg/100 ml)	17 8±4 1	7 4–30 0	9 1±1 9	5.3–12.9
T_3-test, %	53 7±5 6	41 8–66.2	51 6±6.5	35.8–63.3
Free thyroxine index arbitrary units	944±217	389–1581	469±100	294–805
Age hours	39 5±11.3	19–71		
Length cm	51 7±? 1	47–58		
Weight g	4310±492	2450–4150		

MATERIALS AND METHODS

The material comprised 202 full-term infants. All pregnancies had been uncomplicated Blood samples were taken in the morning the second or third day after birth Heel blood was used and 10 to 1? capillary tubes provided sufficient plasma or serum for the analyses In 17 children mixed cord blood was also withdrawn for analyses Total thyroxine determinations were carried out using the Sephadex column method (Tetralute® Ames Company) As routine analyses 50 μl plasma was used but the analyses could be carried out using only 25 μl plasma The unbound TBG binding sites which correlates to the non-proteinbound fraction of thyroxine was evaluated by the T Sephadex retention test (Trilute® Ames Company) This analysis requires a plasma volume of 50 μl The free thyroxine index was calculated as the product of the total thyroxine and the T_3-test values and given in arbitrary units Normal values for euthyroid adult females in our laboratory for the T Tetralute® test. 9 1±1 9 μg per 100 ml (mean ±S D) Normal values for adult females for the T_3-test Trilute® 51 6±6.5% (mean ±S D) Free thyroxine index 469±100 arbitrary units (mean ±S D) (16 17).

RESULTS

The results are given in Table I The mean value of thyroxine in plasma in the newborn was found to be greatly increased compared with normal adult mean values Normal range (95% limits) for full term infants with an average age of 39 hours was 9 6–26 0 μg per 100 ml The mean value of the T_3-test Trilute® was also significantly increased compared with normal adult females ($0\ 01<p<0\ 02$) The free thyroxine index was considerably elevated in the newborn and normal range (95% limits) was 510–1378 No definite peak of thyroxine and free thyroxine values could be demonstrated within the period of investigation which varied from 19 to 71 hours after birth but the highest values were general at the age of 24 hours There was found to be no sex difference in thyroxine and free thyroxine values In cord blood from 17 children mean value of serum thyroxine was 15 6± 2 4 μg per 100 ml (mean ±S D) and the T_3-test mean value 39 2±4 2% (mean ±S.D). The free thyroxine index mean value was in cord blood 612±105 (mean ±S D)

DISCUSSION

In several previous studies of thyroid function in the newborn a hyperfunction has been demonstrated using different thyroid function tests (4 5 6 7 12 13 14 15 18) Most authors agree in finding a definite increase in total plasma thyroxine and free thyroxine in the first two days of life but different maximum values from 3 hours to 24 hours after birth are described (9 10 15) In all previous studies only a smaller number of children have been examined In the present study more than 200 children have been examined with two new micro-thyroid function tests with the purpose of establishing a normal range of total thyroxine and free thyroxine index in the first 3 days of life The micro-thyroid function tests used has previously been correlated to macro-thyroid function tests (12 16 17) The Tetralute T_4-test and the Murphy T_4-test has been found to give practically identical values and the Trilute T_3-test values were highly correlated

to other macro-T_3-tests and to dialysable thyroxine using equilibrium dialyses methods. In our previous study (14) free thyroxine index calculated as the product of Tetralute® and Trilute® has also been correlated with absolute free thyroxine in ng/ml and the coefficient of correlation was 0 92

Normal range for total plasma thyroxine in the present study was found to vary between 9 6–26 0 μg per 100 ml plasma. So far we have a very limited experience with these new thyroid function tests in thyroid disease in the newborn We have had the opportunity of examining one case of congenital hyperthyroidism. This child died the second day after birth and total plasma thyroxine shortly before the death was found to be 32 μg per 100 ml In one case of congenital hypothyroidism serum thyroxine values declined from 8.4 μg per 100 ml the second day of life to 2 2 μg per 100 ml the 5th day of life (19)

The mean value of the T_3-test Trilute® was slightly but significantly elevated compared with euthyroid adults but was greatly increased compared with cord blood values The free thyroxine index in plasma calculated as the product of the total thyroxine content and results of T_3-test is today generally accepted as an estimation of the content of absolute free thyroxine (3 9) Using the free thyroxine index we have confirmed our previous results indicating a thyroid hyperfunction in the neonatal period (15) In this study a rise in free dialyzable thyroxine was demonstrated parallel to the rise in total thyroxine The increase in plasma thyroxine values is thus not explained by an increase in the binding capacity of the thyroxine-binding proteins. In our previous study of free thyroxine in the newborn no significant differences between full-term prematures could be demonstrated as the low total thyroxine concentration in premature infant was matched by a corresponding increase in dialysable thyroxine values In the present study of free thyroxine index therefore only full-term infants have been studied

An early diagnosis of congenital hypothyroidism is regarded as essential to ensure optimal intellectual development (11) We find that a lack of ability to produce the normal physiological thyroid hyperfunction in the first days after birth might be the best indication of congenital primary hypothyroidism The present normal values of total thyroxine and free thyroxine index can only be used in period 19 to 71 hours after birth However if a prospective study of the incidence of congenital hypothyroidism should be carried out this period seems best suited for testing. The physiological increase in TSH in the first hours simulates a TSH stimulation test (20) the newborn are still at the obstetric department and a positive test gives optimal possibilities for early treatment We suggest that the technically very simple micro-thyroid function tests described in the present study are performed on wide indications in the newborn whenever thyroid diseases are suspected. If values of thyroxine or free thyroxine index in the second day of life are found to be without normal range repeated and frequent tests of thyroid function seem to be indicated

ACKNOWLEDGEMENT

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RENAL BICARBONATE REABSORPTION AND HYDROGEN ION EXCRETION IN CHILDREN WITH RECURRENT URINARY TRACT INFECTIONS

The Effect of Fluorohydrocortisone

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ABSTRACT Aperia A., Berg U. and Broberger O (Department of Paediatrics, Karolinska Institutet, S:t Goran s Children s Hospital, Stockholm, Sweden). Renal bicarbonate reabsorption and hydrogen ion excretion in children with recurrent urinary tract infections. The effect of fluorohydrocortisone Acta Paediat Scand, 63:209 1974.—Delayed renal response to ammonium chloride induced acidosis appears to be the earliest detectable residual dysfunction in recurrent urinary tract infections. Control of renal acidifying mechanisms was therefore studied in 11 girls with recurrent urinary tract infections, but with normal glomerular filtration rates. The renal response to ammonium chloride induced acidosis was normal in 7 and pathological in 4 children. Bicarbonate infusion studies demonstrated that the pathological response to the ammonium chloride load was due to depression of the renal bicarbonate threshold. When, however the plasma bicarbonate level exceeded the normal renal threshold, i.e. 24.5 mEq/litre, the reabsorptive capacity for bicarbonate was the same in the patients with pathological and normal ammonium chloride tests. Treatment with fluorohydrocortisone resulted in an increase in bicarbonate reabsorption and hydrogen ion secretion in all patients studied. It is suggested that the low bicarbonate threshold in some patients depends on selective tubular damage in limited number of nephrons. The enhancement of bicarbonate reabsorption by bicarbonate infusion and by fluorohydrocortisone suggests that in the majority of the nephrons renal acidifying mechanisms are intact. It is also suggested that fluorohydrocortisone acts on renal acidifying mechanism mainly by increasing the availability of sodium for exchange with hydrogen ions.

KEY WORDS: Urinary tract infection, ammonium chloride load, bicarbonate reabsorption, mineral corticosteroids

Acidosis is an almost inevitable finding in renal insufficiency It results from impairment both of the ability to reabsorb bicarbonate (19) and to excrete acid products (16) In most patients with renal disease with the exception of some rare tubular disorders laboratory signs of acidosis are uncommon as long as the serum creatinine level is normal Yet in recurrent urinary tract infection when patients with only moderately reduced or even normal glomerular filtration rates are exposed to an acid load a delayed renal response can be detected in a large number of cases (4) The possibility then arises that in this disease there is a progressive impairment of tubular hydrogen ion secretion, but that compensatory mechanisms will afford protection from manifest acidosis until the renal function is reduced to at least 30 %

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Table 1. *Clinical and laboratory data on patients studied*

Patient	Age	IVP	GFR (ml/1.73 m² b.s./min)	Blood pressure (mmHg)	Clinical grouping[d]	Studies: Ammonium chloride load: before florinef	after florinef	Bicarbonate infusion: before	after
A. H.	9	Scarred[b]	99	110/80	C	×		×	
A. J.	13	Scarred[b]	111	110/75	C	×	×	×	×
A. K. B.	10	Small[a]	110	110/70	B	×		×	
A. M.	10	Small[a]	98	105/70	C	×	×	×	
A. W.	7	Scarred[b]	112	110/80	C	×		×	
B. A.	10	Normal	118	105/70	C	×	×	×	×
C. P.	13	Scarred[b]	107	105/70	A	×	×	×	×
I. J.	7	Scarred[b]	102	100/60	C	×		×	
K. O.	8	Normal	93	105/75	B	×		×	
M. B.	14	Small[a]	90	100/65	C	×		×	
M. L.	8	Small[c]	100	105/70	C	×	×	×	×

[a] Pronounced renal parenchymal reduction one kidney. The other kidney normal/hypertrophied.
[b] Scars of renal parenchyma but no significant reduction in size.
[c] One kidney small, one of normal size but with scars.
[d] Clinical grouping:
A. Clinical history of at most one infection with or without significant bacteriuria.
B. Clinical history of one to three urinary tract infections yearly. All those patients have had bacteriuria simultaneous with the symptoms. In none of the patients has there been recurrent episodes of high fever or high abdominal pain radiating dorsally.
C. Clinical history of three or more infections yearly confirmed by bacteriuria or recurrent episodes of high fever and onesided abdominal pain radiating dorsally and simultaneous bacteriuria.

The present study was undertaken to gain some insight into the control of acidifying mechanisms in recurrent urinary tract infection. A quantitative study was made by determination of the capacity to reabsorb bicarbonate during the induction of alkalosis. The capacity to reabsorb bicarbonate was compared with the renal response to an ammonium chloride load which represents only a qualitative test of urinary acidifying capacity.

Mineral corticosteroid hormones could be expected to be of importance for the adaptive control of urinary acidifying mechanisms in man (14). The control of hydrogen ion secretion was therefore re-evaluated in some of the patients after treatment with a standardized dose of fluorohydrocortisone. It is possible that mineral corticosteroid hormones are clinically and therapeutically the most useful of the factors known to influence urinary acidifying mechanisms. A further purpose of this study was therefore to gain some additional information of the mechanism by which mineral corticosteroid hormones influence renal tubular bicarbonate reabsorption and hydrogen ion secretion in man under condition of mild renal disease.

MATERIAL AND METHODS

The studies were carried out in 11 girls 7–14 years old. All the girls had histories of recurrent urinary tract infections. No signs and symptoms of infection or bacteriuria was encountered during the last 3–4 months prior to the study. Negative urine culture 1 month prior to the study and at the time of the study was obtained in each case. Arterial blood pressure was normal in each patient. IVP had been carried out in all girls within 1 year of the study. It was normal in 2 children. Five of the children had signs of slight to moderate renal parenchymal scarring; in 4 of the children there was a marked reduction of the renal parenchyma of one kidney. The glomerular filtration rate was within the normal range in all children. The 24-hour urinary aldosterone excretion was determined in 3 children and was found to be normal. The blood pH, P_{CO_2} and bicarbonate concentration were normal under basal conditions in all patients studied. A clinical summary of the patients is given in Table 1 (1).

All the studies were carried out when the patients were hospitalized in the metabolic ward at S:t Göran's Hospital for Children. From the day of admittance the children received a sodium controlled diet containing 190 mEq sodium/1.73 m² body surface/day. With few ex

ptions the daily urinary sodium and potassium excre- n was determined. Studies of the renal response to an monium chloride load and determination of bicar ate reabsorption during sodium bicarbonate infusion s carried out in all patients under controlled condi- ns. The order between the two studies varied randomly wo days elapsed between the first and the second study. In 5 of the children tests of renal acidifying mech- isms were repeated during administration of fluoro- ydrocortisone (Florinef®). Following the control stud- s the children received fluorohydrocortisone for 6 days . a dosage of 0.4 mg/1 73 m² body surface/day. Dur g the fluorohydrocortisone administration the children lso received a supplement of potassium calculated so at it would exceed the urinary potassium excretion ith 0 5– times. On the 4th day and the 6th day of uorohydrocortisone treatment bicarbonate titration and he ammonium chloride load were repeated. The bi- arbonate infusion study preceded the ammonium chlo- ide study

Ammonium chloride test

A modification of the short time ammonium chloride test described by Edelmann et al. was used (6). In order to keep a constant urinary flow the patients were given water in an amount of 6–10 ml/kg body weight/hour orally. The forced fluid intake was started 2 hours be- fore the intake of ammonium chloride. Urine was col- lected hourly by spontaneous voiding. In the middle of each urine collection period pre-warmed capillary blood samples were taken for the determination of blood pH bicarbonate concentration and pCO_2. Following control sampling of blood and urine ammonium chloride was given orally in an amount of 150 mEq/m² body sur face. After the administration of ammonium chloride another 5 urine samples were collected. Immediately after voiding the urine samples were withdrawn and kept an- aerobically on ice. pH analyses were carried out within 15 min of voiding

Bicarbonate infusion study

The studies were carried out under standardized fluid intake. One hour prior to the start of the first urine collection period the patients received 10 ml water/kg body weight to drink. During the entire course of the study they received 6.5 ml water/kg body weight/hour. Standard clearance techniques were used including con- tinuous infusion of 10 per cent inulin (Laevosan-Gesell- schaft) 0 001 g/min/kg body weight after a prime dose of 0 05 g/kg body weight. The duration of the urine collection periods was 20 min. Urine was collected under oil from a double lumen bladder catheter with one arm connected to a vacuum pump via a urine collecting flask. Urine was drained into the collecting flask during the entire period. Frequently a gentle suprapubic pres- sure was applied. During almost the entire period the other arm of the catheter was clamped. Towards the end of the collection period 2 ml urine sample was withdrawn from the urine collecting flask for analysis of pH. The other arm of the double lumen catheter was then unclamped and the urine collection period was ter minated by gentle suction of the vacuum pump for 30

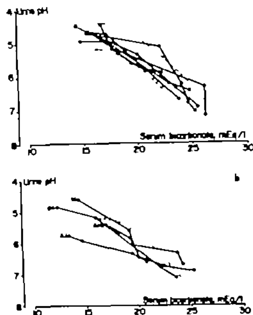


Fig 1 (*a*) Relationship between serum bicarbonate con- centration and urine pH following an oral ammonium chloride load. The area within the dashed lines represents the relationship found in normal children (4). The figure includes the 7 children with normal response. (*b*) Rela- tionship between serum bicarbonate concentration and urine pH following an oral ammonium chloride load. The area within the dashed lines represents the relationship found in normal children (4). The figure includes the 4 children with pathological response

sec. Approximately 5–10% of the urine was collected by suction. In the middle of each urine collection period a venous blood sample was withdrawn for the analysis of inulin and electrolytes and prewarmed capillary blood samples were taken for the determination of blood pH bicarbonate concentration and P_{CO_2}. Following 2–3 control periods an intravenous infusion of 0.6 M sodium bicarbonate was started at the rate of 1 4 ml/1 73 m² body surface/min. This infusion rate was maintained for 4 periods and was then increased to 2 ml/1 73 m² body surface/minute. Following another 3 periods the infusion rate was again increased to 2.6 ml/1 73 m²body sur face/minute. The study was generally interrupted when the serum bicarbonate level rose above 30 mEq/liter

Analytical methods

Analysis of inulin in serum and urine was made with the anthron method (12). Sodium and potassium in serum and urine were determined with flame photometer. Blood pH bicarbonate and pCO_2 were determined with the Astrup method (20). Actual pH of capillary blood samples were determined immediately after the sample had been collected. Additional capillary blood samples were equilibrated with 4 and 8% carbon dioxide gas mixtures and then analysed for pH. All the pH

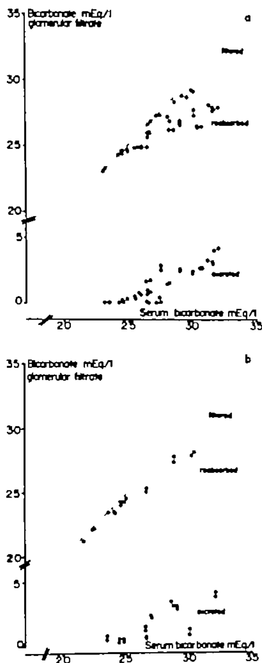


Fig 2 (*a*) The relationship between serum bicarbonate and excreted and reabsorbed bicarbonate All observations from the children with normal response to the ammonium chloride load are included (*b*) The relation ship between serum bicarbonate and excreted and reabsorbed bicarbonate All observations from the children with pathological response to the ammonium chloride are included

determinations were made with a pH-meter 27 (Radiometer) By plotting the pH data on a Siggaard Andersen nomogram P_{CO_2} actual bicarbonate concentration and total carbon dioxide content could be obtained The pH of fresh urine and of urine samples equilibrated with 4 and 8% carbon dioxide gas mixtures were determined with the same pH meter The bicarbonate concentration of urine was calculated by using the Henderson Hasselbach equation assuming the pK of urine to be $6.33 - 0.5\sqrt{B}$ where B represents the sum of urinary sodium and potassium concentrations in equivalents per litre During the ammonium chloride load the actual pH of the urine was determined by a pH-meter 26 (Radiometer) For determination of titratable acidity the urine was titrated with 0 01 ml sodium hydroxide until pH 7 4 In some cases ammonium concentration in urine was analysed by the colorimetric hypochlorite-phenate method (7)

RESULTS

Response to ammonium chloride load

Evaluation of the renal response to ammonium chloride induced acidosis was carried out in all patients During the development of acidosis serial urine and blood samples were collected The individual relationships between blood and urine acidity are illustrated in Fig. 1 *a* and *b* A previous study from this laboratory has established the normal relationships between blood and urine acidity in healthy children (4) In those there is a rapid fall in urine pH when the actual serum bicarbonate level is depressed from 24 to 20 mEq/l. When serum bicarbonate is depressed below 17 mEq/l maximal urine acidity appears to be approached and there is generally little further change in urine pH The level of urine pH then ranges between 4 4 and 5 2 In 7 of the children of the present study the relationship between blood and urine acidity was within normal limits The response of those 7 children is illustrated in Fig 1 *a* In the remaining 4 children that have been included in Fig 1 *b* the relationship between blood and urine acidity deviated from the normal pattern Thus, during the development of acidosis the urine pH was at one or several occasions higher than expected

Response to bicarbonate infusion

Determination of renal bicarbonate excretion and reabsorption was made in all patients during bicarbonate infusion There was no characteristic change of the glomerular filtration rate during the course of the studies Fig. 2 *a* and *b* represent mass plots of reabsorbed

nd excreted bicarbonate at various plasma icarbonate levels The reabsorbed and ex reted bicarbonate has been related to the lomerular filtration rate Fig 3 *a* and *b* il- ustrate the individual relationships between iltered and reabsorbed bicarbonate in patients with normal and pathological response to the ammonium chloride load In Figs. 2 *a* and 3 *a* all observations made in patients with normal response to the ammonium chloride load are included In those patients bicarbonate did not

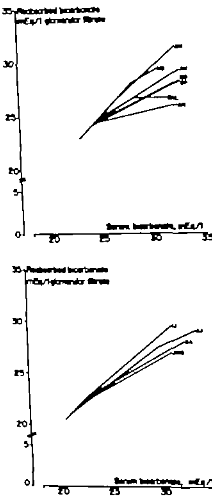


Fig 3 (*a*) Individual relationships between serum bicarbonate concentration and reabsorbed bicarbonate in children with normal response to the ammonium chloride load. (*b*) Individual relationships between serum bicarbonate concentration and reabsorbed bicarbonate in children with pathological response to the ammonium chloride load

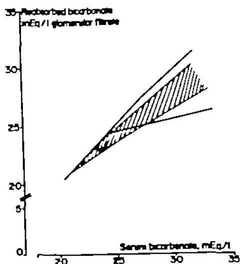


Fig 4 Outer ranges of reabsorbed bicarbonate in patients with pathological response to the ammonium chloride load (dashed area) and in patients with normal response to the ammonium chloride load (area between the two thick lines).

generally start to appear in the urine until the plasma bicarbonate value had exceeded 24 mEq/l In 2 patients bicarbonate did not appear in the urine until the plasma bicarbonate concentration had exceeded 26 mEq/l. The appearance of bicarbonate in the urine however did not mean that the maximal capacity to re absorb bicarbonate was reached In at least 5 of the 7 patients the reabsorption of bicarbonate continued to increase during the study and no real T_m bicarbonate could be demonstrated Figs 2 *b* and 3 *b* include the patients with pathological response to the ammonium chloride load In those 4 patients bicarbonate starts to appear at a somewhat lower serum bicarbonate value When serum bicarbonate concentration is between 21.5 and 24 mEq/l the amount of reabsorbed bicarbonate is definitely lower in the patients with a pathological response to the ammonium chloride load. When serum bicarbonate concentration increases above 24.5 mEq/l there is no apparent difference in the amount of reabsorbed bicarbonate in the patients with normal and pathological response to the ammonium chloride load (Fig. 4) The glomerular filtration rate was

Table 2 *General effects of fluorohydrocortisone*

Patient	Blood pressure (mmHg) Control	Blood pressure (mmHg) Florinef	GFR (ml/1 73 m² b s /min) Control	GFR (ml/1 73 m² b s /min) Florinef	Na balance (mEq/1 73 m² b s.)	Escape"	Basal aldosteron excretion (µg/24 hours)
A J	110/75	110/80	111	108	25	yes	N M
A M	105/70	110/75	98	85	N M	N M	6.3
B A	105/70	100/70	118	127	113	no	3 4
C P	105/70	100/70	107	112	87	no	N M
M L	105/70	125/90	100	121	17	yes	5 9

N M =not measured

slightly higher in the patients with pathological response to the ammonium chloride load (109±5 ml/1 73 m² b s /min) than in the patients with a normal response (100±8 ml/1 73 m² b s /min)

Response to fluorohydrocortisone administration

Five patients received fluorohydrocortisone In 4 of the patients (A J B A C P M L) the response to the ammonium chloride load as well as to bicarbonate infusion was tested during fluorohydrocortisone treatment In patient AM only the response to the ammonium chloride load was studied after fluorohydrocortisone treatment In patient ML BA and CP the steroid treatment resulted in a 20 10 and 5% increase in the glomerular filtration rate respectively In patient A J the glomerular filtration rate was slightly lower and in patient A M it was 15% lower during steroid treatment In all patients the steroid treatment resulted in salt retention initially At the time of the first study however 2 of the patients

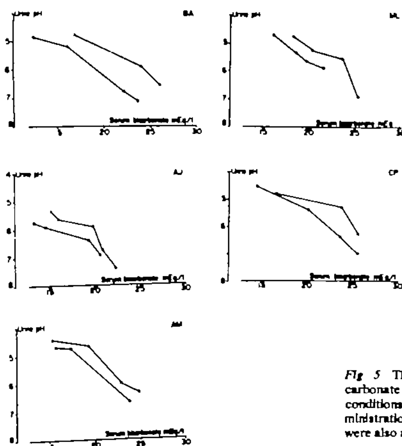


Fig 5 The individual relationships between serum bicarbonate concentration and urine pH during control conditions (●) and during fluorohydrocortisone administration (O) Arrows represent urine samples that were also analysed for ammonia and titratable acid

Table 3 *Effects of fluorohydrocortisone on the excretion of titratable acid and ammonia excretion*

	Titratable acid				Ammonia excretion			
	μEq/1.73 m² b.s./min		μEq/100 ml glomerular filtrate		μEq/1.73 m² b.s./min		μEq/100 ml glomerular filtrate	
Patients	Control	Florinef	Control	Florinef	Control	Florinef	Control	Florinef
A. M	31	45	32	53	36	58	37	68
B. A	30	35	25	28	52	47	44	53
C. P	31	31	29	28	43	61	40	54
M. L	40	34	40	28	31	48	31	40
Mean difference	3.3		0.75		18.0		15.8	
S.D.	8.5		10.03		2.9		10.4	
p	0.5>p>0.4		0.8>p>0.7		0.005>p>0.001		0.1>p>0.05	

(A. J. M. L.) had escaped the salt retaining effect and the daily sodium excretion was not different from what was found during the control days. In all the patients, however, the total salt balance was positive at the time of the first study. There was a definite increase in blood pressure in one of the patients (M. L.). The general effects of fluorohydrocortisone are summarized in Table 2.

Response to ammonium chloride load during fluorohydrocortisone

The effect of fluorohydrocortisone on the renal response to ammonium chloride load is demonstrated in Fig. 5. It is apparent that acidification of urine occurred in a less acid state during fluorohydrocortisone administration. This finding was consistent and observed regardless whether the initial ammonium chloride response was normal (A. M., C. P., M. L.) or not (A. J., B. A.). The effect of fluorohydrocortisone on the excretion of ammonia and titratable acid was examined at the height of urine acidity, i.e. when urine pH appeared to have stabilized at a new low level. The exact points chosen for analysis are shown in Fig. 5. Table 3 demonstrates a statistical analysis of the effect of flourohydrocortisone on titratable acid and ammonia excretion. The parameters were measured in all patients but A. J. The urinary excretion of titrable acid increased in some patients, but the increase was not significant. The urinary ammonia excretion rose consistently and the increase was significant. Since ammonia excretion is related to the glomerular filtration rate (16), the increase might have been due to the increased filtration rate observed during fluorohydrocortisone treatment. However, when the ammonia excretion was calculated per 100 ml glomerular filtrate, the increase during fluorohydrocortisone administration was still significant.

Bicarbonate reabsorption during fluorohydrocortisone

The effect of fluorohydrocortisone on the reabsorption of bicarbonate in patients A. J., B. A., C. P. and M. L. is shown in Fig. 6. To compensate for changes in filtration rate the amount of reabsorbed bicarbonate has been expressed per unit filtered load. It is apparent that fluorohydrocortisone resulted in a consistently increased capacity to reabsorb bicarbonate. At all serum bicarbonate levels above the threshold more bicarbonate is reabsorbed per unit filtered load. The increase in bicarbonate reabsorption occurs regardless whether the glomerular filtration rate has increased more than 5% or not, and regardless whether a salt escape has occurred or not. I occurs in the patients with normal respons

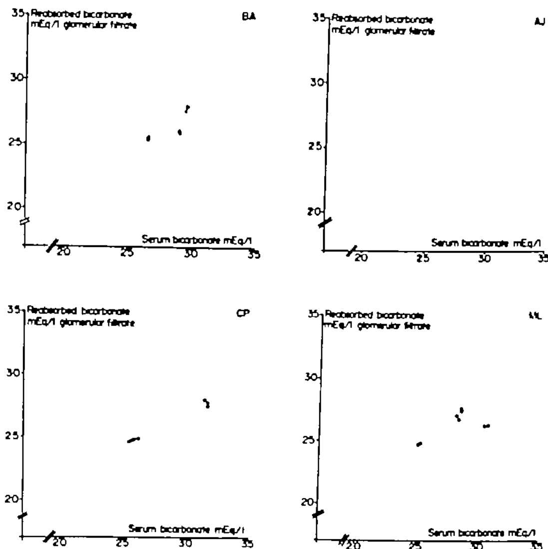


Fig 6 The individual relationships between serum bicarbonate concentration and reabsorbed bicarbonate during control conditions (●) and during fluorohydrocortisone administration (O)

to the ammonium chloride load (C P M L) as well as in those with pathological response (A J B A)

DISCUSSION

It has previously been reported from this laboratory that a large number of patients with recurrent urinary tract infections have a pathological response to ammonium chloride induced acidosis (4) It was suggested that the pathological renal response to the ammonium chloride load mainly depended on a lowered renal bicarbonate threshold Confirming evidence for this suggestion has been presented in this work In the patients with normal response to the ammonium chloride load complete reabsorption of bicarbonate generally occurred until serum bicarbonate exceeded 24 0–24.5 mEq/ In the patients with pathological response to the ammonium chloride load urinary bicarbonate excretion started at a lower serum bicarbonate value

Several previous reports deal with the reabsorption of bicarbonate in renal disease Most of those studies have been carried out in patients with pronounced depression of renal function due to a variety of renal diseases (18, 19 21) The interest has mainly been focused on the maximal reabsorptive capacity for bicarbonate It has been reported that when the glomerular filtration rate is below 37 ml/ 1 73 m² body surface/min the maximal capacity to reabsorb bicarbonate is depressed in relationship to the glomerular filtration rate

21) Little has been known about the renal bicarbonate loss at physiological serum bicarbonate levels in kidney disease In the present study conclusive evidence is presented for a reduced renal bicarbonate threshold in a not negligible proportion of patients with recurrent urinary tract infections but with normal glomerular filtration rates It is hardly likely that the reduced bicarbonate threshold is an immediate and reversible effect of the acute infection since the studies have been carried out at least 2 months generally much longer after the last episode of bacteriuria. The finding of a sign of tubular dysfunction i e incomplete bicarbonate reabsorption in patients with normal glomerular filtration rates is remarkable It is in contrast with the generally held view of the pathophysiology in recurrent urinary tract infection, that diseased nephrons stop to work while the remaining nephrons function homogenously (1 2) This would result in a preserved glomerular tubular balance The glomerular tubular balance of bicarbonate however appears to be frequently disrupted at an early stage in recurrent urinary tract infection. As a matter of fact the glomerular filtration rate was even higher in the patients with low bicarbonate threshold On the basis of this finding it is suggested that increased bicarbonate loss in the urine is the earliest detectable sign of single nephron damage As the damage progresses the nephrons finally cease to function. It is therefore possible that some of the patients in the group with normal bicarbonate thresholds but with somewhat lower glomerular filtration rates represent a later stage in the disease process In those patients some of the nephrons might already have ceased to function while the remaining nephrons are fairly intact.

The low bicarbonate threshold is apparently not a sign of an overall impairment of bicarbonate reabsorption Increases in the filtered load of bicarbonate generally resulted in increases in bicarbonate reabsorption. Actually in most of the patients studied no real T_m bicarbonate could be detected In this respect the patients with low bicarbonate thresholds did not differ from the patients with normal bicarbonate thresholds The fluorohydrocortisone studies present additional evidence that the total reabsorptive capacity for bicarbonate is well preserved in the early stages of recurrent urinary tract infections By the administration of fluorohydrocortisone it was possible to raise the reabsorptive capacity for bicarbonate in all the patients studied As a matter of fact fluorohydrocortisone resulted in an almost linear relationship between filtered and reabsorbed bicarbonate Thus the bicarbonate reabsorptive system could hardly be saturated under basal conditions The fact that the reserve capacity for bicarbonate reabsorption is well preserved even in the patients with pathological response to an ammonium chloride load will explain why manifest acidosis is a rare finding in the early stages of renal disease The loss of small amounts of bicarbonate at physiological serum bicarbonate levels might however in itself have some clinical implications In order to keep the plasma bicarbonate levels constant the patient might mobilize some bicarbonate from the bone (5) The growth might be affected and renal osteodystrophia might be initiated Another consequence to be kept in mind is the more or less constantly alkaline urine which might be a disadvantage in the treatment with certain urinary tract antibiotics

The results from the present study also yield some information on the mechanism by which mineral corticoid hormones affect renal bicarbonate reabsorption and hydrogen ion excretion Although alkalosis is a classical finding in hyperaldosteronism the mechanism by which mineral corticosteroid hormones promote alkalosis is still unclear Some authors ascribe it to a stimulation of sodium reabsorption in exchange for potassium and hydrogen ions in whatever proportions available others believe that it is a phenomenon secondary to potassium depletion (14 8) Some of the difficulties in studying the relationship between the activity of mineral corticosteroid hormones and renal tubular hydrogen ion secretion arise from the

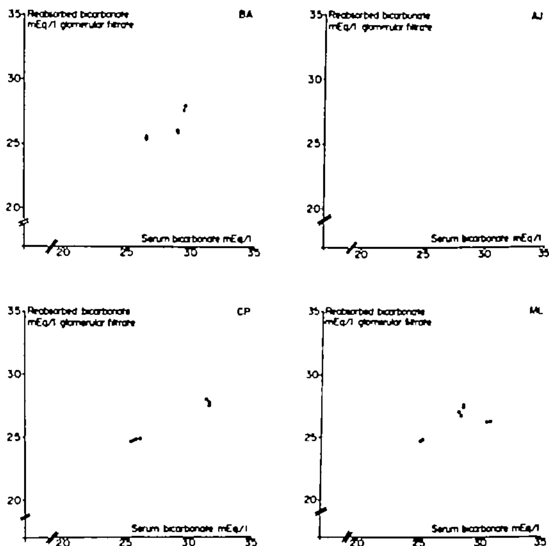


Fig 6 The individual relationships between serum bicarbonate concentration and reabsorbed bicarbonate during control conditions (●) and during fluorohydrocortisone administration (O)

to the ammonium chloride load (C P M L) as well as in those with pathological response (A J B A)

DISCUSSION

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fact that so many related factors also influence hydrogen ion secretion potassium balance (8 18) extracellular fluid volume (13) and blood carbon dioxide tension (15) No previous study has been devoted to the action of mineral corti costeroid hormone on renal acidifying mechan ism in man Mills et al determined the urinary hydrogen ion secretion following the acute in jection of aldosterone and cortison in man and found a general increase (19) Since no attempt was made to relate the hydrogen ion excretion to blood acidity or to any parameter of renal function those studies do not allow any con clusions to be drawn on the direct action of mineral corticosteroid hormones on tubular hydrogen ion transport In dog an enhance ment of bicarbonate reabsorption has been re ported following treatment with desoxycorti costerone acetate (8) This enhancement was suppressed by the dietary supplement of potas sium chloride Those supplements were how ever much larger than in the present study and most likely far in excess of the urinary losses The possibility thus exists that a positive potas sium balance was induced The depression of hydrogen ion secretion might therefore as well be an effect of the positive potassium balance as such In the present study the daily potas sium supplement only exceeded the urinary losses with 0 5–2 times This was thought to be adequate for a maintenance of a constant potassium balance Precautions were also taken to keep the other factors known to effect renal bicarbonate reabsorption and hydrogen ion ex cretion constant All observations were carried out at a normal and constant blood pCO_2 The protocols for the studies during control condi tions and during fluorohydrocortisone treat ment were almost identical with regard to fluid intake and sodium bicarbonate infusion rate It can be assumed that fluorohydrocortisone resulted in a more or less pronounced extra-cellular volume expansion which in turn would act depressing on the tubular bicarbonate re absorption The mechanism by which extra cellular volume expansion inhibits bicarbonate reabsorption is obscure It has been suggested however that the effect is secondary to the inhibition of sodium reabsorption known to oc cur during extracellular volume expansion (10). When hydrogen ions are secreted from the tubular cells the equivalent amount of sodium ions are generally reabsorbed from the tubular lumen in order to keep the electrical gradient constant It is therefore to be expected that renal processes initiated by hydrogen ion secre tion such as the reabsorption of bicarbonate, will be dependent on the availability of so-dium Reabsorption of bicarbonate is thought to take place mainly in the proximal tubule. It is now well established that mineral corti-costeroid hormones also enhance the reabsorp-tion of sodium in the proximal as well as in the distal tubule (11 9) Increased availability of sodium is therefore the likely explanation for the enhancement of bicarbonate reabsorp-tion observed during fluorohydrocortisone treatment It is somewhat remarkable that the enhancement of hydrogen ion secretion and bicarbonate reabsorption was also observed in patients that had escaped from the salt re-taining effect of the steroid hormone In those 2 patients however the sodium balance was still positive The clinical importance of min-eral corticosteroid hormones for the control of acid base balance will probably not be evident until the relationship between sodium and hydrogen ion exchange mechanism has been further clarified

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HISTIDINEMIA AND NORMOHISTIDINEMIC HISTIDINURIA

Report of Three Cases and the Effect of Different Protein Intakes on Urinary Excretion of Histidine

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From the Departments of Paediatrics University Hospitals Umeå and Uppsala and the Department of Nutrition University of Uppsala Sweden

ABSTRACT Holmgren G Hambraeus, L. and de Chateau, P (Departments of Paediatrics University Hospitals, Umeå and Uppsala and the Department of Nutrition, University of Uppsala Sweden) Histidinemia and "normohistidinemic histidinuria" Acta Paediat Scand, 63: 220 1974 —Three patients with histidinemia and one with "normohistidinemic histidinuria" are presented The effects of varying protein intakes on urinary histidine excretion were studied in three of the patients and in 14 controls; histidine excretion in the urine was found to be parallel to an increased protein intake In the histidinuria" patient the urinary histidine excretion was much lower than in the histidinemic patients, but higher than in the controls at different levels of protein intake The occurrence of different phenotypes of histidinemia and renal histidinuria and the value of early dietary treatment is discussed

KEY WORDS Histidinemia 'normohistidinemic histidinuria protein intake

First recognized in 1961 by Ghadimi et al (14) histidinemia is an inborn error of metabolism in which the amino acid histidine cannot be metabolized due to a deficiency of the enzyme histidase

The connection between histidinemia and a specific speech delay described in the first cases has subsequently been questioned (17 25)

Renal histidinuria has also been reported (7 29) This diagnosis however is difficult to evaluate since the excretion of histidine may be influenced not only by the qualitative and quantitative protein intake (6 22) but also by ingestion of phenantoin and related drugs (24) and possibly also by hormones (28)

This study presents the symptomatology in 3 patients with histidinemia and one with normohistidinemic histidinuria in whom the urinary excretion of histidine was studied at different levels of protein intake

METHODS

Qualitative and semiquantitative analyses of histidine in urine were performed by high-voltage paper electrophoresis (HVPE) (20) and the cuprizone reaction (13)

Ion-exchange chromatographic analysis of urine and plasma Ion-exchange chromatographic analyses of deammoniated urine collected in 24-hour portions and deproteinized plasma 50 mg solid sulphosalicylic acid per ml plasma were performed on a Beckman 120 C and/or a Biocal BC 200 automatic amino acid analyser (3)

Histidine loading tests 100 mg L-histidine or 160 mg L histidine hydrochloride per kg body weight was given orally and blood samples were taken at zero 1/2, 1 2, 3 4 6 12 and 24 hours after loading

Dietary preparation The calorie and protein content of the food was calculated for each patient using special Swedish Food Composition Tables (1) and food and beverages were weighed before the meals of all patients

CASE REPORTS

Histidinemia

Case No 1 (M B 70 02 17) A girl the third of three siblings No known consanguinity Her eldest sister is patient No 2 Mental retardation or neurological disease is not known in the family

Table 1. *Urinary excretion of histidine, 1-methylhistidine, 3-methylhistidine and carnosine in case No. 4 and his relatives*

Values are given in µmoles/24 hours

	Case No. 4	Mother	Father	Maternal grand-mother	Maternal grand-father	Normal values
Histidine	2 100	2 300	940	1 320	1 160	213–1 490[a]
1-CH_3-histidine	310	221	67	76	129	15–1 510[a]
3-CH_3-histidine	91	26[illegible]	–	227	235	
Carnosine	87	226	59	245	190	
Urine volume ml/24 h	560	800	1 200	900	1 700	
Renal histidine clearance ml/min/1.73 m^2	21.0			–	–	9.5±2.6[b] 9.3±6.8[c]

(3–65 y.) given as 95% limits (9).
(2–13 y.) given as mean ±S.D. (5).
(7–13 y.) given as mean ±S.D. (21).

Birth weight 3 850 g. An elevated blood histidine level was found in the national infant blood screening program at 6 weeks of age. The patient was admitted at the age of 22 months for verification of the suspected diagnosis of histidinemia. Physical and psychomotor development were found to be normal. She had no speech defect. Testing according to Vineland and Gesell was normal.

Calculations from 24-hour dietary recalls revealed a self-restricted protein intake of about 1 g per kg body weight. She did not like dairy products and eggs and sometimes refused to eat protein-rich food.

Case No. 2 (H. B. 59 04 24). A girl, the first of three siblings. Histidinemia was diagnosed during the family investigation of Case No. 1. Birth weight 3 980 g. Checked regularly at child health centres. Physical and psychomotor development normal. Normal school performance and no speech defect at the age of 13 years.

Case No. 3 (H.-R. J. 47 09 18). A male. A half-brother was institutionalized for psychosis of unknown nature. Otherwise little is known about psycho-neurological diseases in the family.

Birth weight 3 000 g. Early psychomotor performance was normal. Poor school performance. Admitted at the age of 16 years because he had lost interest in his usual activities, had difficulty keeping up in school and had been observed to walk in a peculiar slow manner. IQ was 79 (Terman-Merrill). A specific speech defect was present. Physical examination was normal. Histidinemia was diagnosed during a metabolic screening (26).

Normohistidinemic histidinuria

Case No. 4 (S. B. 55 10 15). A boy, the only child of healthy parents. A maternal cousin suffers from epilepsy. As shown in Table 1, a family investigation of urinary amino acid excretion revealed that the mother of this patient also had a somewhat increased urinary excretion of histidine on repeated examinations. The father and the maternal grandparents showed normal urinary excretion of amino acids. The paternal grandparents were dead.

Birth weight 3 080 g. Early psychomotor performance normal. Healthy until the age of 13 years when frequent myoclonic seizures with impairment of consciousness appeared. Anticonvulsive drugs had no effect. Physical examination and testing according to Terman-Merrill at the age of 13 years were normal. Died at the age of 15 years through drowning.

Pneumo-encephalography, vertebral angiography, electromotor conduction velocity in the ulnar nerve and eyeground examination were all normal. EEG showed a generalized abnormality, pronounced during sleep and on photoactivation. No further changes in the EEG-recordings were seen during peroral loading with L-histidine. The cerebrospinal fluid showed normal cell counts and protein content.

Intravenous pyelography was normal. The maximal osmolar urine concentration was 1 040 mOsm/litre.

An increased urinary excretion of histidine was detected by the metabolic screening (19). The renal clearance of histidine was found to be slightly increased, as shown in Table 1.

RESULTS

Verification of diagnosis

As indicated in Table 2, an increased urinary histidine excretion was observed in all 4 patients. The plasma histidine level was elevated in patients Nos. 1–3 but normal in patient No. 4. As shown in Fig. 1, the histidine loading test was abnormal in cases No. 1 and No. 3, i.e. there was an initially steep elevation of the plasma histidine concentration and a slower decrease than in the 2

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Table 2 *Plasma concentration and urinary histidine excretion in histidinemia cases 1–3 and in normohistidinemic histidinuria case No 4 and corresponding data reported in the literature*

	Case 1 (22 months)	Case 2 (12 y)	Case 3 (23 y)	Case 4 (13 y)	Normal values Mean±S D (7–13 y)[a]	Histidinemics range[b]
Plasma (µmoles/l) Histidine	463	770	310	90	97±39	300–910
Urine (µmoles/g creatinine) Histidine	6 830	6 970	6 560	1 510	970±350	1 610–14 190

[a] (21) [b] (16)

control persons The mother of patients Nos 1 and 2 (C B) showed a higher plasma histidine concentration after 12 hours than did the control persons which could indicate a heterozygous state of histidinemia In the histidinuria patient case No 4 the loading test was normal

Studies on the influence of dietary protein intake on urinary histidine excretion in the patients

As seen in Fig 2 the urinary histidine excretion was higher than that of the controls in all cases when expressed as µmoles histidine per g creatinine This increase was far more pronounced in the histidinemic cases

DISCUSSION

Many problems concerning histidinemia and histidinuria such as diagnostic criteria symptomatology occurrence of different phenotypes and the distinction between histidinemia and histidinuria are still unsolved Similar problems are still under discussion concerning hyperphenylalaninemia and phenylketonuria (18)

The variation in symptomatology of histidinemia is exemplified by comparison of cases Nos 1 och 2 with case No 3 While the latter had a speech defect and progressive mental retardation the former were normal at the ages of 3 and 14 years respectively This raises the question whether these cases may represent different phenotypes or different diseases The difference in symptomatology was not correlated to plasma histidine levels or urinary excretion of histidine since these were higher in the asymptomatic patients Nos 1 and 2 than in the severely affected patient No 3

The influence of treatment on the prognosis

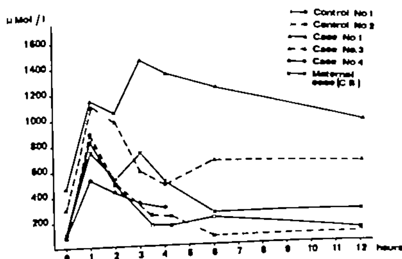


Fig 1 Plasma histidine levels during the peroral histidine loading test in the histidinemic patients (cases 1 and 3), the normohistidinemic-histidinuria patient (case 4) the mother of patients 1 and 2, and in 2 normals.

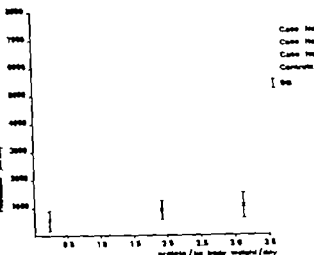


Fig. 2. Effect of different protein intakes on the urinary histidine excretion (μmoles histidine per g creatinine) in 2 cases of histidinemia (cases 1 and 3), 1 patient with "normohistidinemic histidinuria" (case No. 4) as well as in 14 control children (21).

of histidinemia diagnosed in newborns is uncertain and so far there is little evidence that a protein- or histidine-restricted diet is of benefit in histidinemia (8, 12, 15 and 25). The significance of the self-restricted protein intake of patient No. 1 is open to discussion.

Histidinuria has earlier been reported associated with an increased excretion of anserine, carnosine, 1-methylhistidine and 3-methylhistidine in cases with cerebromacular degeneration and convulsions (4). In our case No. 4 only an increased urinary excretion of histidine was found on several occasions (Table 1). Furthermore no cerebromacular degeneration was seen.

Isolated defects in the renal reabsorption of amino acids, at times associated with neurological symptoms, have earlier been reported, i.e. cystinuria (23) and glycinuria (27). Whether there is a connection between the renal histidinuria and the convulsions in our case is open to discussion. The EEG-changes observed in this patient, however, were of the same type as those seen in diffuse encephalopathy with epilepsy, also called the Lennox syndrome (11).

The demonstration of increased histidine excretion in the urine in two successive generations in our case No. 4 suggests a dominant mode of inheritance. Interestingly, Galamond et al. (10) reported a family in which histidinuria was associated with stammering in three successive generations, also consistent with autosomal dominance. Their patients were initially presented as cases of histidinemia, but analyses by means of ion exchange chromatography performed at another laboratory (2) revealed histidinuria, but normal plasma histidine values.

Incidental observations of histidinuria have also been reported in different surveys (7, 29), but since plasma levels were not given, and no considerations were given to dietary protein intake, hormones or anticonvulsive medication, they are difficult to evaluate. Furthermore, no psycho-neurological symptoms were present in these cases.

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FAMILIAL OCCURRENCE OF CORNELIA DE LANGE S SYNDROME

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ABSTRACT Beck, B. (Paediatric Department TG, Rigshospitalet, Copenhagen, Denmark) Familial occurrence of Cornelia de Lange's syndrome. Acta Paediat Scand, 63:225 1974.—A family is presented in which two sibs, brother and sister exhibit Cornelia de Lange' syndrome The mother and younger sister present certain peculiarities characteristic of the syndrome. The case speaks in favour of a genetic etiology and modes of inheritance are discussed.

KEY WORDS: Cornelia de Lange's syndrome familial occurrence

Cornelia de Lange s syndrome which was first described in detail by the Dutch pediatrician Cornelia de Lange in 1933 has during the last decade attracted special interest in the medical literature Most cases have occurred sporadically and as only a few sibships with more than one affected member have been described it has been meant of interest to present a sibship in which the two eldest children present the syndrome in a most typical form in which the next born child is normal and in which finally the youngest child *present certain anomalies often seen in Cor* nelia de Lange s syndrome

The two probands were first seen by the author in 1966 and since then examined by the author at intervals The two younger children were both seen by the author at their birth and since then followed at intervals Clinical examinations and X-rays mentioned here were performed during September 1972

CASE REPORTS

Sib no 1 (proband) a boy C P (Fig 1), born September 22nd 1962, was first presented at a Danish Paediatric Society Meeting (Warburg & Puerregaard (16)). The pregnancy was complicated by vaginal bleeding in the seventh month after an abdominal trauma. The mother reported feeble movements of the fetus. The child was born at term in breech presentation with the umbilical cord once around the neck and was slightly asphyctic. Birth weight 2 600 g, birth length 50 cm and head circumference 31 cm. The placenta was described as small membranes and umbilical cord as normal The first years were char acterized by frequent infections He was able to sit alone "very late" and walked unsupported 2 years old. Since the age of 1 year 3 months he has been an institutionalized patient. IQ (Cattell) 1963 63 1966 35

From the age of 4–5 months to 15 months he suffered from daily convulsions of the upper extremities. At 9 months old the EEG was found normal, the PEG showed atrophia cerebri with medially enlarged ventricles and abundant surface air At 18 months old, horizontally placed acetabuli and elongated colon sigmoideum were found

Personal examination 1972 showed very unconcentrated and restless 10-year-old boy with looks char acteristic of Cornelia de Lange s syndrome (Table 1). The speech was bad he was able to pronounce but a few words. Height 134 cm, weight 24 6 kg and head circumference 48 cm. Average height and weight for age: 138.5 cm (S.D 5 7) and 32 1 kg (S D 4 4) (14)

Laboratory investigations showed normal fractionated serum proteins and serum lipids, protein-bound iodine and serum thyroxine Acid mucopolysaccharides (AMP) (age 6 years) showed a fraction corresponding to chondroitin-sulphate repeated analyses at age 9 years showed elevated excretion of AMP presumably keratosulphate (Dr J Clausen, Neurochemical Institute). Further investigations—see Table 1

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Table 1. Findings of a family with two sibs C. P. and B. P. exhibiting Cornelia de Lange's syndrome

+ symptom present 0 symptom absent – missing information.

	O P	R. E. P	C. P	B. P	R. P	M. P	H. R.
Growth failure							
intra-uterine	–	–	+	+	0	0	0
in later life	0	0	+	+	0	0	0
Mental retardation							
mild IQ 75–50	0	0			0	+	0
moderate IQ 50–21			+	+			
severe IQ below 20							
Characteristic physical appearance							
nose	0	0	+	+	0	0	0
mouth	0	0	+	+	0	+	0
ears	0	0	0	0	0	0	0
micro-/brachycephaly	0	0	+/+	+/+	0	0	0
hirsutism of back and extremities	0	0	+	+	0	+	0
low hair line of forehead and neck	0	0	+	+	0	+	0
long, thick curly eyelashes	0	+	+	+	0	+	0
synophris	0	+	+	+	0	+	0
clinodactyly of fifth fingers	0	+	+	+	0	0	0
single flexion crease of fifth finger	0	0	0/+	0	0	0	0
proximally placed thumbs	0	0	+	+	0	0	0
severe extremity malformations	0	0	0	0	0	0	0
undescended testes/hypospadia	0		+/+		0	0	0
nystagmus/strabismus	0	0	0/+	0	0	0/+	0
major structural eye defects	0	0	0	0	0	0	–
Chromosome analyses	0	0	0	0	0	0	–
X-rays							
broad first metacarpal	–	–	+/+	+/+	–	0	–
malformed phalanges of fifth finger	–	–	+/+	+/+	–	0	–
elbow malformations	–	–	+/+	0	–	0	–
micro-/brachycephaly	–	–	+/+	+/+	–	0	–
Galactose 1-phosphate-uridyl-transferase units/g Hb	27.9	30.2	31.9	31.8	30.8	36.4	–

once or twice daily. The birth was uncomplicated. Weight 4 400 g, height 55 cm and head circumference 34 cm. Placenta, membranes and umbilical cord were normal. The child made no problems neonatally. Yet her looks were peculiar. The hair grew low into forehead and neck and there was abundant lanugo, esp. of the back. The first fingers appeared short, but there were no evident limb malformations. The superciliary bow was long and curved, the eyelashes long and abundant. The lips thin and crescent shaped with a wide space between nose and lips. The neck short and thick.

The early development was normal. She was vaccinated against pertussis 5 and 9 weeks old without immediate complications. From the age of 8 weeks she had series of convulsions with flexion of the extremities without loss of consciousness. At the age of 2–3/4 months the EEG was heavily abnormal showing epilepsy and focal traits, but gave no suspicion of hypsarrhythmia. She was treated with corticotropine and cortisone with diminishing frequency of attacks. The EEG was improving. After dismission from hospital she was treated with Phenytoin. No attacks have been seen since the age of 12 months. At 24 months old the IQ (Cattell) was about 70 with marked retardation of the language.

Personal examination 1972 (Table 1): height 95 cm, weight 15.4 kg, head circumference 47.5 cm. Average height and weight for age: 85.8 cm (S.D. 4.1) and 12.4 kg (S.D. 1.3) (14).

Laboratory investigations showed normal fractionated serum proteins and serum lipids. T_3-test, serum thyroxine and urine amino acids. For further investigations see Table 1.

The mother R. E. P. (Fig. 4) was born on June 26th 1941. From childhood she was hampered by low back pain caused by spondylolisthesis vert. lumb. V. Her IQ was 77 (Binet-Simon). In 1959 she was admitted to a psychiatric ward because of neurosis anxiosa somatisata with abundant tics. 4 pregnancies were carried through 1962, 1963, 1968 and 1970. According to earlier statements she had had 7 abortions. In 1971 she remembered only 3 early abortions between child no. 2 and 3. Somatic



Fig 1 C P 4 years old

Sib no 2 (proband) a girl B P (Fig. ?) born December 31st 1963 The pregnancy was complicated by slight vaginal bleeding in the 5th and 7th months The child was born at term in head presentation. Birth weight ? 800 g length 49 cm and head circumference 32 cm The placenta umbilical cord and membranes were described as normal From the third week the child had repeated vomitings which gradually diminished. She was prone to infections She sat alone 1 year old walked unsupported 2 years old and said her first words ? years 4 months old IQ (Cattell) 1964 60 1968 32

She is still living at her home with daily attendance at a special kindergarten Convulsions have never been observed and EEG at age 12 months has been normal

Personal examination 1972 showed a somewhat aggressive unconcentrated and restless girl with looks characteristic of Cornelia de Lange s syndrome (Table 1). She was able to pronounce a few words Height 125.5 cm weight 24 kg head circumference 49 5 cm Average height and weight for the age 13? cm (S D 5 7) and 28 6 kg (S D 4 3) (14)

Laboratory investigations showed normal fractionated serum proteins and serum lipids normal protein-bound iodine and T_3-test Urine for acid mucopolysaccharides showed nothing abnormal Further investigations see Table 1

Sib no 3 a boy R P born February 11th 1968. Pregnancy and birth uncomplicated placenta membranes



Fig 2 B P 5 years old.

and umbilical cord normal. The mother had 5 mg diazepam daily during pregnancy The appearance of the child was normal at birth weight 4 100 g length 54 cm and head circumference 35 cm The early development was normal At the age of 1 year the IQ (Cattell) was 80 with special retardation of the language

Personal examination 1972 showed a normal 4½ year-old boy (Table 1) Height 11 cm, weight ?6 kg, head circumference 5? 5 cm Average height and weight for age 10?.5 cm (S D 4 8) and 16 6 kg (S D 1 8) For further investigations see Table 1

Sib no 4 a girl M P (Fig. 3) born on February 14th 1970 During pregnancy the mother had diazepam 10 mg



Fig 3 M P 6 months old

…rnal					
…angles	Total	Simian crease		Fifth finger flexion creases	
33	66	0	0	2	2
36	77	0	0	2	2
57	111	+	+	2	1
48	93	+	0		2
38	90	0	0	2	2
41	104	0	0	2	2



Fig 4 R. E. P 23 years old.

favour of a common etiology maybe an environmental factor in uterine life maybe a genetic factor No specific environmental factor has been found in the three pregnancies. The placenta of the first child C P has been described as small whereas the placentas of the other children all have been described as normal

The mother seems to have had a conspicuous number of abortions but no examinations have been made of these

The age of the parents at the birth of the eldest child was 21 and 24 years at the birth of the youngest child they were 28 and 32 years old. No consanguinity was found In the father s family were found no cases of mental retardation The father himself was one of a pair of presumably monozygous twins of which the other one died 10 months old of unknown cause In the mother s family two paternal cousins were mentally retarded neither of these with remarkable looks. The possibility of recessive inheritance exists in a family with two cases of Cornelia de Lange s syndrome However no consanguinity has been found in this family or in other familial cases Further cases of the syndrome have not been found in the family

Several workers have stressed the occurrence of some of the characteristics of the syndrome in near relatives in favour of a genetic etiology Keizer (8) described a typical Cornelia de Lange patient. One brother of the patient exhibited brachycephaly another "starlike eyelashes and a sister had syndactyly Apart from these other malformations occurred in the mother s family

Borghi (4) described a family in which consanguinity was known and where the father and his mother presented clinodactyly and proximally placed first fingers and where a cousin of the father was very like the patient

Beer et al (2) described a sibship of 8 children of which the proband was number 7 Among the other children 4 exhibited facial traits very like those of the proband

Among the patients described by Abraham & Russell (1) one had an elder brother who died shortly after birth exhibiting several of the symptoms characteristic of the syndrome.

To elucidate further the possibility of formes fruste of the syndrome further in-

Table 2 *Dermatoglyphic patterns of the hands of a family with two sibs C P and B P exhibiting Cornelia de Lange s syndrome*

A arch U ulnar loop R radial loop T tented arch + pattern present 0 pattern absent

		Fingertip patterns											
		Left					Right						
Case	Sex	V	IV	III	II	I	I	II	III	IV	V	Total ridge count	Z p- dt pres
O P	M	U	U	U	R	W	W	R	U	U	U	149	0 0
R E P	F	U	U	U	R	U	U	U	U	U	U	66	0 0
C P	M	U	U	R	R	U	U	U	U	U	U	33	+ +
B P	F	U	U	R	R	U	U	R	U	U	U	106	+ 0
R P	M	U	U	A	A	W	U	A	A	U	U	69	0 0
M P	F	U	U	U	R	U	U	R	U	U	U	63	0 0

examination height 178 cm weight 70 kg The physiognomy was dominated by heavy eyebrows meeting centrally the eyes laterally set the palpebral fissures normal For further investigations see Table 1

The father O P was born on September 15th 1937 He attended normal school At the age of 33 years he weighed 67 kg was 181 cm high His looks were unremarkable somatic examinations showed no abnormalities For further investigations see Table 1

Because of doubt of paternity with regards to M P examination of blood groups (ABO rhesus NMS P Le K and Fy) were made The blood groups of all children were in accordance with the assumption of the paternity of O P

Sib no 5 a boy H R born on September 22nd 1972. The clinical examination of this half-brother revealed no signs of illness or likeness to the other children (Table 1)

DISCUSSION

This characteristic syndrome has most often been described as sporadic cases of which till 1970 about 250 cases have been registered (3) Only very few familial cases have been reported

The first family with more than one affected member was mentioned by Ptacek et al (12) They described an affected boy with a sister who had died 11 years previously with the characteristic looks of the syndrome Motl & Opitz (10) in their survey describe four sibships known i e Ptacek et al (12) Opitz et al (11) one sibship with 2 affected sisters and 3 presumably affected brothers who were either stillborn or had died neonatally and another sibship with one affected boy and an affected miscarriage of 8 months with typical traits Finally reference to the sibship of this communication is made Three sets of monozygotic twins are known all of them being concordant for the syndrome namely the pair mentioned by Opitz et al (11) by Choo & Branchi (5) and by Kroth (9) The last-mentioned twins appear to have typical physiognomies whereas the malformations of the extremities with polydactylia and broad endphalanges of first fingers usually are not seen in Cornelia de Lange s syndrome In the sibship of 4 children presented here are found 2 children very much alike and exhibiting most characteristics of Cornelia de Lange s syndrome As another sib M P presents some of the characteristics of the syndrome (Table 1) it would be of interest to look for these symptoms in the ascendency too The looks of the father are quite unremarkable, whereas the eyes and their surroundings of the mother exhibit certain peculiarities. On scrutiny of photos of the mother in her early childhood and of photos of her parents it must be concluded however that the heavy eyebrows and somewhat laterally set eyes which are found in the mother and in her mother as well cannot be considered as unusual

The abnormal clinical findings which C P B P and M P have in common speak in

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vestigations have been undertaken (Table 1) Chromosome analyses (Dr Margareta Mikkel sen the Kennedy Institute) have shown nor mal karyotypes in all family members Galac tose 1 phosphate uridyl-transferase (Dr A Dahlquist Lund) which in Cornelia de Lange patients has been found elevated (7) was ele vated in the mother and all 4 children The highest level (36 4) was found in M P then 10 months old By the same method Dahlquist et al (6) found a mean value of 28 1 units/g Hb with a SE of 0 57 in 40 normal subjects

X rays of C P B P and M P showed malformations characteristic of the Cornelia de Lange syndrome in C P and B P whereas M P showed normal head hands and elbows (September 1972) At an earlier examination M P showed microcephaly radiologically

Finally dermatoglyphics have been made in 6 family members (Table 2) Total ridge counts were found ranging from 33 to 149 In a material of 1 079 Danish males and 1 075 Danish females Vogelius Andersen (15) found a mean total ridge count of 138 17 $\pm$ 1 52 and 129 35 $\pm$ 1.52 According to this ridge counts of the mother and all 4 children seem rather low The characteristic zygodactylous configuration described in Cornelia de Lange patients by Smith (13) is seen in both hands of C P and in the left hand of B P Only in these three hands are simian creases seen On the right fifth finger C P presents but one flexion crease The distribution of the patterns of the finger tips is shown in Table 2 Both patients have radial loops on the second and third fingers of the left hand and B P has a radial loop on the second finger of the right hand

According to the clinical description and the above mentioned further investigations a concept of 2 patients with Cornelia de Lange s syndrome and a sister having a forme fruste of the syndrome is still to be taken into consideration

Hitherto no progeny of a Cornelia de Lange patient has been seen It would however be interesting to learn if any of the aforementioned relatives with minor malformations have had children exhibiting Cornelia de Lange s syndrome or other malformations

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FLUORINE CONCENTRATIONS IN DECIDUOUS HUMAN TEETH AFTER ORAL ADMINISTRATION OF SODIUM FLUORIDE IN VITAMIN SOLUTION

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ABSTRACT Bervenmark, H and Hamberg L. (ACO Läkemedel AB, Solna and the Department of Paediatrics Karolinska sjukhuset Stockholm, Sweden) Fluorine concentrations of sodium fluoride in vitamin solution Acta Paediat Scand 63 232, 1974.— In an earlier study 705 children were given a vitamin A and D solution; of these 342 received a supplement of sodium fluoride over a period of 7 years. A significant difference in the frequency of caries was found between the children in the fluoride group and those in the control group To see if this difference was correlated to the concentration of fluorine in enamel and dentine a quantitative analysis of deciduous teeth from both groups was carried out The results indicate that fluoride administered orally in the dosage used significantly increases the fluoride content of the enamel and dentine The findings might contribute to explain why children who receive this prophylaxis have increased resistance to caries.

KEY WORDS Caries, fluoride

In an effort to reduce the incidence of caries a controlled trial was initiated in the spring of 1959 *in which children were given sodium* fluoride in vitamin solution at Stockholm City Child Welfare Centres

Seven hundred and five children took part in a double blind study 342 in the fluoride group and 363 in the control group Over a period of 7 years they were given a vitamin A and D solution daily with or without a supplement of sodium fluoride containing 1 1 mg sodium fluoride (10 drops=0 5 mg fluoride) The children in the study group were thus assured a daily dose of fluoride corresponding to that in 1 liter of water containing 0 5 ppm fluoride Examination of the children s teeth at 3 4 5 and 6 years old showed a 50% lower frequency of caries in the fluoride group (2 3) As the difference between the fluoride group and the control group was so great we considered it necessary to make a quantitative fluoride analysis of deciduous teeth from the *two groups to see if there was any relation* between orally administered fluoride and fluoride concentrations in enamel and dentine In this analysis only deciduous upper and lower front teeth without caries were used

METHODS

The teeth were first pounded in a specially constructed plunger and cylinder apparatus made of iron, and then pulverized in an agate mortar The separation of enamel and dentine was made according to Manly & Hodge (4) The diffusion separation of fluoride ions was carried out in polyethylene stoppers using Baumler s (1) method and the fluoride determinations were made using a specific ion meter Orion Research Ionalyzer" model 401 and the fluoride electrode 94-09

The separation of enamel and dentine was made exactly as described by Manly & Hodge (4) using a bromoform–acetonemixture (91+9 vol% density 2 70) Enamel and dentine were then weighed and put into the thoroughly washed polyehtylene stoppers (size B 34) Wash-

Table 1 *Ppm fluoride found in enamel and dentine samples from the two groups of children*

	Fluoride group		Control group	
	Enamel	Dentine	Enamel	Dentine
	172	110	95	–
	135	78	–	108
	114	–	105	–
	119	–	–	61
	161	–	–	101
	–	177	60	71
	–	136	–	81
	–	117	28	44
	68	177	58	76
	–	155	57	67
	–	174	51	58
	81	183	45	49
		131	57	75
	112	237	67	84
	79	170	65	103
		103	–	112
	–	113	71	93
	96	158	45	–
			33	–
		187	29	–
		175	–	–
	–	131	–	–
	99	205	–	–
	114	167		
		174		
	85	182		
	91	173		
	76	167		
	91	147		
	88	119		
	74	151		
	18	27	15	15
$\bar{x}$	103	155	58	79
s	29	35	22	21

ing of stoppers and all other equipment is essential to avoid errors caused by fluoride residues. The following cleaning procedures were used polyethylene stoppers and covers were put into a 10% sodium hydroxide solution for hours and then placed in nitric acid 5 M overnight, rinsed with demineralized water and dried in a drying cabinet at 60°C All other glass vessels were rinsed first with tap water then with demineralized water and placed in nitric acid 5 M overnight Rinsing and drying were carried out in the manner described above. Pipettes were rinsed with a chromic-sulphuric acid mixture and alcoholic potassium hydroxide solution (70 g KOH per 1 000 ml alcohol) and finally rinsed in demineralized water and dried as above.

All equipment was rinsed with distilled water immediately before use and then dried. The samples of enamel and dentine were weighed with the polyethylene stoppers together with 3 ml distilled water (glass apparatus) and 2.25 ml perchloric acid (70%) cooled with ice. A circular filter paper (Munktell 1F) impregnated in the centre with 0.1 ml sodium hydroxide solution 3 N was placed in the upper recess of the stopper The filter paper was fixed by a polyethylene ring placed upon the paper and held in place by the silicone-greased cover The stoppers were then kept at 55°C for 48 hours. Subsequently the filter papers were transferred to flasks and shaken in a microid shaker for 10 min together with 20 0 ml acetate buffer solution pH 5 6 and 20 0 ml distilled water (glass apparatus). The determinations of fluoride ion were made directly in the solutions without removing the filter papers.

The reliability of the measurements and the working procedure was tested by treating known amounts of sodium fluoride in the exact same manner as the samples. We have also added fluoride to previously analysed enamel and dentine samples and have found that the recovery is better than 95% if the working procedure described above is carefully followed.

Acetate buffer solution pH 5.6 48 ml acetic acid 0.4 M and 452 ml sodium acetate solution 0.4 M

Standard solutions: various amounts of a stock sodium fluoride solution were mixed with a 50.0 ml acetate buffer solution (pH 5.6), 0.1 ml sodium hydroxide (3 N) and distilled water to 100 ml A standard curve was made for the concentration range 0 1–10 0 ppm fluor

RESULTS

The following results expressed as ppm fluoride were obtained for the enamel and dentine samples from the two groups (Table 1) Teeth from a total of 31 children in the fluoride group and 23 children in the control group have been analysed Some of the results have been deleted for different reasons. In some cases the samples of enamel and dentine have been too small in other cases the results obtained have been low or near the limit of accuracy of the method and hence not reliable. This was true especially of the control group It might have been desirable to use this fact in the statistical evaluation in order to get a higher significance on comparing the averages. As far as we know however there are no statistical methods that permit missing results to be used If such methods had been available we should have had a better basis for our conclusions In this connection it should be mentioned that the person making the determinations did not know which samples came from the fluoride group or the control group and could not omit or influence the results

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BRAIN STEM ENCEPHALITIS

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ABSTRACT Yalaz, K. and Tinaztepe K. (Departments of Paediatric Neurology and Paediatric Pathology Hacettepe Children's Hospital, Ankara, Turkey). Brain stem encephalitis. Acta Paediat Scand 63: 235 1974.—Since the clinical picture in brain stem encephalitis resembles that of various other neurological diseases and conditions, especially brain stem gliomas, and since the prognosis and treatment of these disorders differ greatly accurate diagnosis is very important. Eight cases of brain stem encephalitis are presented; seven with signs indicating good prognosis, and the post-mortem findings of one fatal case. Analyses of the clinical and laboratory findings of our cases allowed the following conclusions:

1 There is a prodromal period of fever and general malaise before neurological symptoms appear

2. Dysphagia, peripheral facial paralysis and pyramidal tract signs are usually present.

3. Although the clinical condition is grave at the onset of illness, the prognosis is good; in 7–8 weeks complete recovery may occur but facial nerve paralysis may persist.

4. Lymphocytes may be present in the cerebro-spinal fluid along with slight increase in protein.

5. Distortion of the fourth ventricle which is of extreme importance in the diagnosis of brain stem gliomas is also usually encountered in brain stem encephalitis.

KEY WORDS: Brain stem encephalitis

Acute encephalitis occurs after non-specific infections and is quite commonly seen during childhood Although the brain is diffusely involved in most cases of viral encephalitis focal involvement may occur On rare occasions ataxia, tremor and nystagmus occurs when there is involvement of the cerebellum (16) Infrequently pure cranial nerve palsies and pyramidal tract disturbances are the most prominent findings In 1951 Bickerstaff & Cloake published a report of three cases with the title of Mesencephalitis and Rhombencephalitis (1) Later in 1957 Bickerstaff added five more cases to his original study and named the condition brain stem encephalitis" (?)

Because brain stem encephalitis may show similar neurological signs to those seen with brain stem glioma (8 9 13) acute bacterial or tuberculous meningitis (15) Reye s Syndrome (12) intoxications (11) myasthenia gravis (10) encephalomyelo-radiculitis (4 5) or Fisher s Syndrome (7) and since the prognosis and treatment of these disorders differ accurate diagnosis is very important This communication presents eight cases of this clinical syndrome with signs indicating good prognosis and the post-mortem findings in one fatal case Brain stem encephalitis could only be diagnosed by post-mortem examination and positive viral studies

In this report post-mortem findings in one case and clinical neurological and prognostic similarities in the other seven patients suggested brain stem encephalitis despite an absence of viral studies

DISCUSSION

If the averages of the enamel fluorine content are compared by using the t test we get $t = 5.1$ which corresponds to $p < 0.0005$ The corresponding calculation for dentine is $t = 8.8$ Hence the differences in fluoride content in enamel and dentine between the fluoride group and the control group are significant If we compare the averages within the two groups we find a significant difference between the fluoride content in enamel and dentine in the fluoride group but not in the control group This may support the concept that fluorine enters the enamel from the inside when fluoride is administered orally in a high enough concentration The results have shown that when fluoride is administered orally in this dosage the fluoride content of the enamel and dentine increases significantly The findings provide an explanation for the increased resistance to caries found among the children offered this prophylaxis This chemical analysis has confirmed the findings of the previous clinical studies

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Case 2. A 5-year-old boy was admitted to our hospital with chief complaints of fever lasting for 20 days, dysphagia and right hemiparesis of 2 days duration. On physical examination, the child was conscious. Facial and soft palate palsies and Babinski signs were present bilaterally. Muscle reflexes were hypoactive. The findings of pneumoencephalography and carotid angiography were normal. EEG revealed findings consistent with diffuse encephalopathy. During his stay in the hospital, the child's neurological signs and symptoms showed some regression. Two months after admission slight hemiparesis remained.

Case 3. A 5-year-old boy was brought to the hospital with complaints of fever, cough, dysphagia and convulsions for the past 5 days. The child was unconscious, there was nuchal rigidity. Left facial palsy and bilateral soft palate palsies were present. Muscle reflexes were hypoactive. Positive Babinski sign was elicited bilaterally. EEG revealed diffuse dysrhythmia. These pathological symptoms and signs gradually regressed. A year after admission slight paralysis was still present on the left side of the face.

Case 4. A 2 ½-year-old girl was brought to the hospital with complaints of fever, convulsions and drowsiness lasting for 3 days. On physical examination the child was unconscious. Left peripheral facial palsy was present. Fundoscopic examination revealed no abnormality. Muscle reflexes were hyperactive and positive Babinski signs were present bilaterally. EEG revealed diffuse dysrhythmia. Findings of the pneumoencephalography and carotid angiography were within normal limits. While in the hospital the neurological signs of the patient seemed to regress. Eight months after admission, follow-up examination revealed only slight peripheral facial palsy on the left side.

Case 5. A 6-year-old boy was admitted to the hospital with chief complaints of fever, abdominal pain, difficulty in swallowing and no micturition for 4 days. The child was conscious but suffered from a soft palate paralysis. Muscle reflexes were hyperactive. Babinski signs were present bilaterally. The left side of the patient's body showed hypoaesthesia. EEG findings were within normal limits. A week after admission, pneumoencephalography revealed left cortical atrophy. The child was discharged in 2 weeks with residual hemiparesis on the left side.

Case 6. A 3 ½-year-old boy was admitted to the hospital with chief complaints of fever, vomiting, difficulty in swallowing and weakness in the extremities lasting for 4 days. The child was conscious. Both fundi were normal. Soft palate and left facial paralysis were present. Muscle reflexes were hyperactive. The Babinski sign was absent. The EEG revealed dysrhythmia involving the left hemisphere. A month after admission, follow-up examination revealed the presence of left facial paralysis.

Case 7. A 4-year-old girl was brought to the hospital with chief complaints of fever, vomiting and difficulty in swallowing of days duration. The child was conscious, there was no nuchal rigidity. Both fundi were normal. Left facial paralysis and soft palate paralysis were present. Muscle reflexes were within normal limits. Babinski sign was negative bilaterally. EEG revealed dysrhythmia involving the left occipital area. Two months after admission all abnormal neurological signs were absent and the child appeared to be completely well.

Case 8. A 3-year-old boy was admitted to the hospital with chief complaints of drowsiness, dysphagia and fever of 3 days duration. In the hospital the drowsiness was found to be intermittent. There was a right facial paralysis. Muscle reflexes were hypoactive and Babinski sign was negative. The right carotid angiogram was normal and the EEG showed diffuse dysrhythmia. Two weeks after admission the child was discharged with very slight facial paralysis on the right side.

FINDINGS

Eight cases of brain stem encephalitis ranging in age from 2.5 to 8 years were diagnosed at Hacettepe Children's Hospital in a three year period. Common prodromal signs were fever of 39–40°C in all patients and dysphagia and drowsiness in some. All patients were brought to the hospital within 2–30 days of the onset of symptoms. Four patients had urinary retention. During the first 2–3 days in the hospital drowsiness and unconsciousness regressed. Unilateral facial weakness due to the seventh nerve involvement, dysphagia due to involvement of the ninth and tenth cranial nerves and paralysis was present in 7 out of 8 patients. Paralysis of other cranial nerves, notably the sixth, was not present. Two patients showed convulsions at the onset of disease. Gait disturbances were present in all except one patient. Muscle reflexes were normal in one, hyperactive in three and hypoactive in four. A Babinski sign was elicited bilaterally in five cases. After a short period in the hospital the neurological signs regressed in all cases except one. Facial paralysis continued in 4 patients, 2 showed hemiparesis and one recovered completely.

LABORATORY FINDINGS

Cerebro-spinal fluid (CSF) opening pressure was normal in all cases. The cell count ranged between 10–210 lymphocytes per cubic mil





Fig. 1. Section of brain stem showing oedema, neuronal degeneration and perivascular cellular cuffing. (Haematoxylin-eosin ×75)

Fig. 2. Perivascular mononuclear cellular infiltrate under higher magnification. (Haematoxylin-eosin ×150).

Fig. 3. Neuronal degeneration and glial node formation under high magnification. (Haematoxylin-eosin, ×150).

CASE REPORTS

Case 1. A boy aged 11 was admitted to Hacettepe Children's Hospital with chief complaints of fever, drowsiness and difficulty in breathing. Two weeks prior to admission dysphagia and dysarthria were noted. Sore throat had been present for the last 3 days. Nephritis had been diagnosed 5 months previously. Family history was not contributory. The patient was drowsy with minimal response to painful stimulations. The eye movements were not impaired and the fundi were normal. Muscle reflexes were hypoactive. Soft palate and right peripheral facial nerve palsies, quadriparesis and bilateral Babinski signs were present. The mouth was filled with secretion and respiration was difficult. Laboratory findings were as follows: Hb 11.7 g/100 ml, white cell count 15000/mm with prominence of neutrophils and abundant thrombocytes in the differential count, urine analyses were within normal limits. NPN 35 mg/100 ml, CO_2 23 mEq/l, Na 135 mEq/l, K 5.7 mEq/l, Cl 97 mEq/l, serum glutamic oxaloacetic transaminase 35U, serum glutamic pyruvic transaminase 12U. Lumbar puncture revelaed a pressure of 140 mmH_2O, no cells, protein 61 mg/100 ml and sugar 96 mg/100 ml, simultaneous blood sugar was 146 mg/100 ml. On X-ray examination routine skull series, chest and plain abdominal films were all within normal limits. Electroencephalogram (EEG) showed slow wave activity in the left hemisphere.

The patient was given parenteral fluid. On the second day of admission, a Bird respirator was employed to ease severe respiratory difficulty. Despite all efforts, haematemesis occurred on the third day and the patient died.

Post mortem examination was confined to the brain. On gross examination, the brain weighed 1345 g (normal weight for this age is 1275 g). Pia arachnoid membranes were found to be transparent with engorged veins. Gyri were flattened and the sulci were narrow. The brain stem appeared enlarged and swollen and was more prominent on the left side. The Circle of Willis and the cranial nerves did not reveal any pathological changes. Coronal sections of the brain showed good demarcation of the white and grey matter. Lateral ventricles were narrow. On serial sectioning, no tumour was seen in the brain stem. The floor of the fourth ventricle was displaced posteriorly. Microscopic examination of several sections taken from each level of the brain stem revealed perivascular round cell infiltration (Figs. 1 and 2), irregularly distributed neuronophagia, glial star and node formations (Fig. 3) and evidence of acute myelin breakdown. No intracellular inclusions were detectable. There was no morphological evidence of any demonstrable fungi or bacteria. Sections other than the brain stem showed non-specific changes consistent with brain oedema. Although no virus-resultant inclusions were observed, some type of viral etiology was most likely.

ploration On the other hand if there is displacement of the fourth ventricle and if symptoms persist for about 8 weeks after the onset of illness, clinical diagnosis of an infiltrative brain stem glioma is most likely and radiation therapy is almost definitely indicated Posterior displacement of the fourth ventricle was present in one of our cases in which post mortem study of the brain was possible In three cases in our series no abnormality of the location of the fourth ventricle was observed with pneumoencephalography one week after the onset of the disease

Tuberculous meningitis should also be considered in the differential diagnosis Tuberculomas in the brain stem produce disturbances similar to brain stem encephalitis (15) Generally in cases of tuberculous meningitis, once drowsiness develops it progresses consistently and CSF examination reveals high levels of protein with increased numbers of cells and markedly decreased level of sugar

Other neurological diseases enter into the differential diagnosis Organic phosphorus intoxications can be excluded when low levels of cholinesterase activity are present in the blood (11) The clinical features of acute onset of myasthenia gravis resemble those of brain stem encephalitis (10) These can be easily excluded however by the edrophonium chloride test. Encephalo-myelo-radiculoneuropathy which is acute inflammatory or allergic reaction involving both the central and the peripheral nervous system include a variety of cases (14) The diagnosis is usually suspected because of an afebrile course the early onset of facial palsy the associated radicular involvement of the lims and the cell protein dissociation in the cerebro-spinal fluid (5) Fisher syndrome is associated with total ophthalmoplegia, ataxia and areflexion It is an unusual form of idiopathic polyradiculoneuropathy associated with a benign course and resembles brain stem encephalitis to a great extent (7)

Complete recovery occurs in most cases of brain stem encephalitis (1) Apparently the pathologic process does not produce permanent tissue damage in the affected area. On post-mortem studies non-specific histological findings such as edema anoxic neuronal changes and degeneration of Purkinje cells are observed Chronic myelin breakdown and chronic astrocytic reactions are not usually present (3) There are perivascular cellular cuffing, glial nodes glial stars and neuronophagic reactions all of which are consistent with viral encephalitis (14)

In our patients even though viral studies were not performed a viral etiology is suspected It is very unusual for any etiological agent to be isolated Recently however Herpes virus hominis were isolated from 2 patients with brain stem encephalitis (6)

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Table 1 *Laboratory findings*
N D = Not done N = Normal

	Cerebro-spinal fluid studies					Blood chemistry		
Case no	Pressure	Cells lymphocytes per cubic ml	Bacteriological culture	Protein (mg/100 ml)	Sugar (mg/100 ml)	Simultaneous sugar	SGOT (U)	SGPT (U)
1	N	None	No growth	61 120	96	146	35	12
2	N	None	No growth	28 22	40	90	48	?4
3	N	None	No growth	34 95	78	45	N D	N D.
4	N	160	No growth	35 21	53	57	N D	N.D.
5	N	None	No growth	30	7?	N.D	1?	1?
6	N	?10	No growth	36 4?	48	98	?5	25
7	N	10	No growth	72 30	70	100	N.D	N.D.
8	N	7	No growth	?0 35	48	72	N D	N D

limeter In 3 patients CSF protein and sugar levels were within normal limits A week after admission the protein level was 95–120 mg/100 ml in 2 cases SGOT and SGPT levels were normal (Table 1) Routine skull films were normal Pneumoencephalography was performed in 3 cases In one patient the left ventricle was slightly dilated The fourth ventricle was normal in all 3 cases EEG showed diffuse dysrhythmia and slow wave activity in 5 patients while one showed no abnormality

DISCUSSION

As pointed out in a previous publication the development of functional disturbances due to brain stem encephalitis occurs at any stage during the subacute course of any viral illness (1 2) Bickerstaff summarized the clinical course and findings of his 8 cases as follows Gradual onset severe lethargy ophthalmoplegia with other cranial nerve deficits and minimal long tract signs (2) In general at the onset of this disease the clinical condition of patients is so grave that it is difficult to predict the probability of recovery Although widespread involvement of the brain was present in these patients symptoms and findings due to brain stem lesions was most prominent and obscured other signs. Since the clinical picture in brain stem encephalitis resembles that of brain stem glioma, accurate differential diagnosis is of the utmost importance A long and progressive course is usually elicited in patients with brain stem glioma (8 9) However in the advanced stage of brain stem encephalitis, evaluation by neurological examination alone may not be adequate for diagnosis The presence of pyramidal tract signs in most cases of brain stem glioma increases the difficulty in differentiating this disease from brain stem encephalitis

Posterior displacement of the aqueductus of Sylvius and/or the fourth ventricle which was thought to be diagnostic of brain stem glioma and/or space occupying lesions in this location can also be seen in cases of brain stem encephalitis (13) Therefore it is necessary to be cautious before starting radiation therapy in cases of brain stem glioma where the diagnosis depends merely on clinical and radiological examination without surgical ex

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INFANTILE ANEURYSM OF THE DUCTUS ARTERIOSUS

Diagnosis Incidence Pathogenesis and Prognosis

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ABSTRACT Heikkinen, E. S., Similä, S., Laitinen, J and Larmi, T (Departments of Surgery Paediatrics and Roentgenology University of Oulu, Oulu, Finland). Infantile aneurysm of the ductus arteriosus. Acta Paediat Scand, 63:241 1974.—A neoplastic-like density on X-ray is not a rare diagnostic problem in infancy Nevertheless, knowledge about the infantile ductal aneurysm (IDA) producing a characteristic tumour-like mediastinal shadow on plain chest film is very limited

In 1972 the authors operated two newborns because of left-sided tumour-like mediastinal shadows produced by IDA A very similar radiographic mass according to location, shape and opacity could be discerned retrospectively on the chest films of 10 other neonates in material of 1 130 newborn babies, who had been radiographed mainly for respiratory troubles. Apparently the IDA is not so rare as considered in the literature

The patients presented herein were found during their first day to have inadequate ventilation, a low Apgar score and respiratory acidosis. Therefore postnatal hypoxaemia was considered the most probable primary factor in the pathogenesis of IDA.

A follow-up examination at the age of 6–24 months showed that the typical tumour like mediastinal shadow had disappeared also in the non-operated patients. A spontaneous, uncomplicated involution seems to be the most usual outcome of IDA. However according to the literature IDA is a potentially dangerous abnormality It calls for meticulous observation since surgical intervention is indispensable if fatal complications seem imminent.

KEY WORDS: Infantile ductal aneurysm, mediastinal tumour

The true spontaneous infantile ductal aneurysm (IDA) is considered rare (3 6) This abnormality occurs in early infancy and means a fusiform dilatation of the ductus with a patent aortic end and a completely or incompletely obliterated pulmonary end (3 23) Only 57 IDAs have been reported up to date (17) However the IDA is not all so rare as the aneurysm of the ductus arteriosus in childhood or in adults (3)

A correct antemortem diagnosis of IDA has never been established (6) excluding two operatively verified lesions These operations were done by us in 1972 because of tumour like density in the plain chest film (12) Many authors have pointed to a mediastinal extra mass produced by ductal aneurysm in children or in adults (3 4 8 11 21 22, 24 33) but only Scheef (29) and Knerdel (18) have shown that also the IDA can be seen as a characteristic shadow in plain chest film The findings of our operated patients correspond well with the observations of Scheef

Prompted by the characteristic roentgenographic sign in the two operatively treated infants we found retrospectively 10 further

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Table 1 *Groups of radiographic findings*

Type of finding	No. of patients	Per cent
Normal	897	79
Thymic hyperplasia	136	1
Visible aortic arc	85	7
Ductal aneurysm infiltration	1	1
Non-classified infiltration in the region of examination	8	1
Total	1 138	100

Findings of the inspected chest films

The chest radiograms of the material on analysis were divided into 5 groups (Table 1)

The majority was composed of the radiograms in which the left upper mediastinum the silhouette of the large blood vessels and the left pulmonary field gave no cause for commen

In 12% a widening of the upper mediastinum typical of thymic hyperplasia was visible

Visualization of a normal aortic arc was noted in 7%

In less than 1% of the material indefinite and atypical infiltration that could not be classified was visible in the inspected area It might have been due to thymic hyperplasia atelectasis of a segment of the left upper lobe hyperplasia of the lymph nodes dilatation of the large blood vessels or possibly a dilated ductus arteriosus

The chest radiograms of 10 neonates showed a finding which in our opinion corresponded completely in site shape and opacity and approximately in size to that on the radiograms of the operated aneurysms used as control material (see Figs 3–5) Hence the radiographic ductal aneurysm group consisted of 12 patients (3 girls and 9 boys all newborn (Table 2)





Fig 3 Left radiogram (Case 1) shows characteristic shadow of IDA. Right radiogram shows how the neonatal shadow has spontaneously disappeared in the follow-up X-ray



Fig 1 The preoperative chest radiogram of the operated patient (Case 9) shows the mass caused by the ductal aneurysm adherent to the upper mediastinum

IDAs in neonatal chest films On the basis of the analysis of the 12 patients we surveyed the incidence diagnosis pathogenesis and prognosis of the IDA

PATIENTS AND METHODS

The total of newborns treated on the neonate wards of the Department of Pediatrics University of Oulu between January 1 1970 and June 30 1977 was 5 500 Chest roentgenograms had been taken of 1 138 of these at an age of less than 1 month These roentgenograms were re-inspected The most common indications for roentgenography had been asphyxia aspiration infection, RDS hyperbilirubinaemia anomalies and prematurity No oblique views or oesophagography were included. Only an antero-posterior and direct lateral radiogram were available

The radiographed patients included the two whose ductal aneurysm was verified and resected operatively (1?) The shadows seen in these patients preoperative radiograms were first defined as carefully as possible. The other chest films in the material were then inspected for similarly defined shadows, typical of the ductal aneurysm

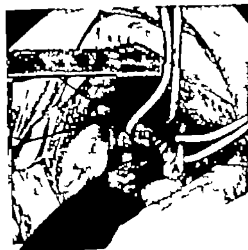
The prenatal and immediate postnatal condition of the patients with a characteristic mediastinal shadow was checked from the case journals and the patients were followed up with examinations that included chest radiography

RESULTS

Verified lesions

In the silhouette of the aortic arc and the pulmonary cone both of the two operatively verified aneurysms (Fig 1) had caused a distinct mass of soft opacity and with a convex contour projected in the left pulmonary field not visualized in a direct lateral radiogram A slight deviation of the trachea to the right could be observed in both cases

The operative explorations were in full agreement with the radiographic finding. They revealed 2 0–2 5 cm long and 1 6 cm wide ductal aneurysms with a fusiform expansion and dark walls and filled with un organized thrombus (Fig 2)



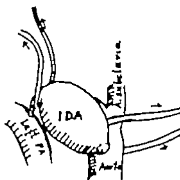


Fig 2 The photograph (Case 9 shows the intraoperative situation The fusiform aneurysm (*IDA*) descending aorta (*A*) trunk of the left pulmonary artery (*P*) vagus nerve (*V*) and the recurrent nerve are exposed On the right side schematic drawing of the photograph on the left side (PA=pulmon

Table 3. *Acid-base balance in the blood of the newborn with radiographic ductal aneurysm*

Patient no.	Acid-base balance							
	During the first hour of life				*At the age of 4 hours*			
	pH	P_{CO_2}	HCO	BE	pH	P_{CO_2}	HCO^-	BE
1	7.15	[illegible]	19	−8.0	7.39	28	20	−5.8
2	–	–	–	–	7.48	77	23	−1.0
3	7.22	54	19	−6.8	[illegible]	[illegible]	20	−5.5
4	7.30	44	20	−5.4	–	–	–	–
5	7.20	51	17	−10.9	7.30	48	[illegible]	−5.0
6	7.31	39	19	−7.5	7.33	29	19	−8.0
7	7.22	51	14	−15.0	7.41	22	20	6.0
8	7.23	[illegible]	19	−8.0	7.31	43	20	−5.3
9	–	–	–	–	7.32	37	19	−7.0
10	7.22	64	18	−7.8	7.31	42	20	−7.0
11	7.21	50	17	−10.6	7.28	37	18	−9.3
12		–	–	–	–	–	–	–
Mean	7.23	57.2	18.0	−8.9	7.34	37.5	20.0	−5.9
±S.D.	±0.05	±24.3	±1.7	±2.7	±0.07	±11.2	±1.3	±2.2

57.2±24.3 significantly higher ($p<0.01$) than for normal babies of this age (pH 7.33 and P_{CO_2} 36.1±4.[illegible]) (19). Respiratory trouble was the indication for radiography apart from one patient (Case 3). No significant murmur could be heard by normal auscultation from the heart of any of the patients.

Follow-up observations

The follow-up examination revealed that one patient of the aneurysm group had died in a local hospital of peritonitis produced by ileus. This patient had Turner's syndrome. The other patients, both the operated and the unoperated, were subjectively and objectively well. Radiography failed to show in any of them the ductal shadow which had been visible at the first radiographic examination (see Fig. 3).

DISCUSSION

Site, size, shape, density

The reliability of the radiographic diagnosis based on a plain X-ray picture depends on how characteristic the lesion is according to its site, size, shape and opacity. With ductal aneurysm the site and opacity seem to be particularly characteristic.

The 2 operated patients in the present material (12) showed beyond dispute that the ductus arteriosus, when dilated into an IDA, is not totally covered by the mediastinal shadow in the antero-posterior view, but may produce a tumour-like extra shadow. A similar observation was reported by Scheef in 1939. The post mortem angiographic reproduction by Kneidel (18) shows that IDA is best visualized in a right oblique view.

According to literature the maximum length of IDAs ranges from 2.5 to 1.5 cm and the maximum diameter from 0.9 to 1.6 cm (1, 2, 5, 6, 12, 18, 23). The size of the infiltrates may therefore vary considerably.

The shape of the diagnosed aneurysms has been constant. According to descriptions they have been oval, fusiform or egg-shaped. Usually the middle part has been widest and the end of the aortic side somewhat wider than the junction with the pulmonary artery (3, 1[illegible]).

Practically always the aneurysm has con-

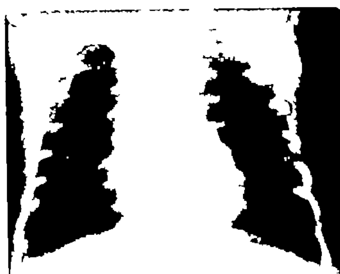


Fig 4 The neonatal radiogram from Case 11 showing the characteristic tumour-like shadow of IDA



Fig 5 The neonatal radiogram from Case 12 showing the characteristic tumour like shadow of IDA

Clinical and laboratory data of neonates with IDA

Only 5 of the group had had normal delivery (Table 2) Their birth weights (3 411±520 g) did not differ from the mean of North Finnish children (3 444±569) (25) The Apgar score at the age of 1 minute ranged from 1 to 9 (mean ±S D 7 2±2 4) which differed significantly (p<0 001) from the corresponding Apgar score of Finnish children (15). During the first hour of life the acid-base balance of their blood without exception suggested respiratory acidosis (Table 3) The pH of the capillary blood 7 23±0 05 was significantly (p<0 001) lower and the P_{CO_2}

Table 2 *Some clinical data on the newborn with radiographic ductal aneurysm*
N=Normal V=vaginal B=Breech presentation VE=Vacuum extraction C=Caesarean section

Patient no	Sex	Gestation (in weeks)	Delivery	Birth weight (g)	Apgar scores (1 min/15 min)	Voice	Indication for radiographic examination	Other coincidental disease
1	M	38	N	2 710	8/8	Shrill	Respiratory distress	Pneumothorax dx
2	M	40	N	4 200	9/10	Normal	Aspiration	Hyperbilirubinaemia, Fract of the left clavicle
3	F	38	B	2 300	9/10	Normal	Goitre	Thyreotoxicosis cong (LATS)
4	M	36	VE	3 500	8/8	Normal	Asphyxia	–
5	F	39	VE	3 800	5/9	Normal	Asphyxia intrapart	–
6	M	39	N	3 280	7/8	Normal	Asphyxia intrapart	Hypocalcaemia
7	M	41	C	3 600	5/9	Normal	Asphyxia antenatal	Prolonged infection
8	F	38	V	3 250	9/9	Normal	Aspiration	Turner's syndrome Coarctation of aorta
9	M	39	V	3 600	9/9	Hoarse	Aspiration	–
10	M	36	C	3 170	7/9	Hoarse	Aspiration	–
11	M	41	C	4 050	1/9	Normal	Asphyxia	Cerebral haemorrhage
12	M	40	N	3 470	9/9	Normal	Aspiration	–

all whose immediate postnatal course had been normal

Prognosis

It is evident that the prognosis in the major ity of IDAs without treatment is good unlike the aneurysm of the adult group and the ar terial aneurysms in general The abnormal dilatation of the lumen is accompanied by necrosis of the intima and the resulting thrombosis of the lumen (23) The organization of the thrombus can usually lead to uncomplicated conversion of the IDA into ligamentum arteriosus (2, 20 23 27) The present observations support the assumed spontaneous involution of infantile aneurysm The literature however provides evidence that aneurysmal malformation of the ductus in the newborn may also lead to fatal complications such as rupture (3 11 29) dissecation (2 3 16) embolisation (3) or phrenic paralysis (1) For this reason a shadow characteristic of ductal aneurysm is a significant finding in a neonatal chest radiogram and requires the attention of the pediatrist radiologist and surgeon In our opinion surgical exploration should be made if the mass is large if it seems to be persisting or growing, or if complications caused by adjacent tissues are associated.

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tained a fresh unorganized thrombus (3) The radiographic opacity of the lesion results from the wall and the thrombus It is characteristically slight and corresponds to the density caused by the large vessels This explains why the aneurysms were not visualized in the lateral views It also explains why infantile aneurysm has by no means always been detected in chest radiograms (1 16 32)

Differential diagnosis

Differential diagnosis is important in the intra thoracic lesions of the newborn which in site size shape and opacity are reminiscent of these aneurysmal shadows A number of solid tumours are possible in the left upper me diastinum teratoma neurogenic tumours thymoma lymphomas lymph node metasta ses and the enterogenic cysts (9 13 14) The spontaneous involution among the non-opera ted cases of the present material excludes the above possibilities Hemangioma hygroma and intrathoracic goitre are different in shape from the aneurysm The differential diagnosis is probably most difficult between aneurysm of the aorta and the pulmonary artery (14) or inflammatory lymph node hyperplasia (29) Aneurysm of the aorta and the pulmonary artery is usually accompanied by an ausculta tory murmur but this was not observed in any of the present patients Moreover spon taneous involution is hardly possible in these aneurysms Inflammatory lymph node hyper plasia usually involves the hilar nodes They are therefore commonly located more cau dally than the ductal aneurysm and are firmly associated with the hilar shadow

Angiocardiography may be the examination of choice to confirm the diagnosis (16) The aortic wall can be seen to give a marking of the duct although IDA wold be totally occlu ded by thrombus in most cases

Clinical symptoms

Not once has an IDA been suspected on the basis of clinical symptoms The case journals of our 3 patients of the aneurysm group mentioned a hoarse or shrill voice This can be explained as a relatively specific symptom of the ductal aneurysm arising from a stretching of the recurrent nerve (1 7 12 30 32)

Pathogenesis

Normally the initial closing of the ductus arteriosus takes place by muscular contraction 10–15 hours after birth when the oxygen pressure increases This is followed by obliteration of the duct without thrombus formation in 2–3 weeks (10 28) Postnatal hypoxaemia of the newborn especially the low birth weight prematures can prevent the muscular contraction of the duct or cause an either partial to total re-opening of the contracted duct (17 28) The etiological mechanism of IDA may consist of a persistent contraction of the pulmonary end and the patency or re-opening of the other parts of the duct The indications for which chest radiograms were taken in the aneurysmal group of the present material the acid–base balance during the first hour of life and the low Apgar score at the age of 1 minute distinctly suggested respiratory trouble and the possibility of hypoxaemia Similar observations of immediate postnatal cyanosis flaccidity and resuscitative measures are common in case reports concerned with IDA (6 16)

Incidence

Thore (31) reported a high frequency of aneurysm in an autopsy material of the new born He found 8 aneurysms per ca 1 000 autopsies Similarly Rauchfuss picked out his disputed 12 IDAs from 1 400 consecutive autopsies (26) These chance findings from routine autopsies provide support for the present results am ong which the aneurysm group became unexpectedly large Earlier newborns disorders for which reason the aberration due to hypoxaemia in the involution of the ductus in autopsy materials may have received added emphasis Accordingly the present material was selected to comprise all the neonates with respiratory difficulties excluding

all whose immediate postnatal course had been normal

Prognosis

It is evident that the prognosis in the major ity of IDAs without treatment is good unlike the aneurysm of the adult group and the ar teral aneurysms in general The abnormal dilatation of the lumen is accompanied by necrosis of the intima and the resulting thrombosis of the lumen (23) The organization of the thrombus can usually lead to uncomplicated conversion of the IDA into ligamentum arteriosus (2 20 23 27) The present observations support the assumed spontaneous involution of infantile aneurysm The literature however provides evidence that aneurysmal malformation of the ductus in the newborn may also lead to fatal complications such as rupture (3 11 29) dissecation (2, 3 16) embolisation (3) or phrenic paralysis (1) For this reason a shadow characteristic of ductal aneurysm is a significant finding in a neonatal chest radiogram and requires the attention of the pediatrist radiologist and surgeon In our opinion surgical exploration should be made if the mass is large if it seems to be persisting or growing, or if complications caused by adjacent tissues are associated

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DIETARY TREATMENT IN HYPERPROLINAEMIA TYPE II

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ABSTRACT Similä, S. (Department of Paediatrics, University of Oulu Oulu, Finland). Dietary treatment in hyperprolinaemia Type II Acta Paediat Scand 63:249 1974 — Hyperprolinaemia type II was diagnosed in a half-year-old boy (proband) with convulsions and an encephalitis-like period, and his symptomless brother aged 7 years. The plasma levels of proline were greatly elevated 39.8 and 38.4 mg/100 ml, respectively The urinary excretion of free proline and Δ-pyrroline-5-carboxylic acid (5PC) was increased. Reduction of the dietary proline of the proband after the first encephalitis-like period at the age of 7 months resulted in a slight decline of the plasma proline level and of the urinary excretion of proline and 5PC. After a 14-month dietary period convulsions began to recur and an encephalitis-like period followed. Thereafter with a more restricted proline (90 mg/kg per day) intake the decline of the plasma level of proline was clear During a 3 years period of dietary treatment the patient's growth and mental development were satisfactory but the electroencephalogram abnormalities remained. Restriction of dietary proline in the hyperprolinaemic brother resulted in prompt fall of the plasma level of proline. The restriction of proline however did not normalize the elevated plasma level of proline and the urinary excretion of 5PC did not cease completely These results suggest that endogenous synthesis is not an important source of circulating proline Restriction of dietary proline in hyperprolinaemia type II may be necessary during infancy at least if the plasma level of proline is greatly elevated as in our proband.

KEY WORDS Amino acids, diet, inborn error of metabolism, mental retardation, proline

Hyperprolinaemia appearing in several members of the family was first described by Schafer in 1962 (22) Subsequent studies (8) revealed two separate types (Fig. 1) Both are characterized by elevated blood proline levels and increased urinary excretion of proline hydroxyproline and glycine In type II furthermore Δ pyrroline 5-carboxylic acid (5PC) can be detected in the urine

Hyperprolinaemia type II has been described in five families (4 9 13 25 28) This inherited inborn error of metabolism is associated with abnormal electroencephalogram and occasionally with mild probably progressive mental retardation (9 13 24) convulsions (4 9 13 24 25) and encephalitis-like periods (13 24 25)

The plasma proline in man may be derived from dietary protein decomposed collagen and the "non-essential" amino acids glutamic acid and ornithine along the pathway indicated in Fig. 1 The enzymes necessary for its synthesis differ from those involved in its degradation (16) Short term feeding experiments of adults have shown that a positive nitrogen balance can be maintained on a proline free diet (20) which is why this amino acid is generally regarded as "non-essential"

I have previously reported two children with hyperprolinaemia type II (28) A detailed clinical description and pedigree of these patients and their family has appeared elsewhere (25)

The purpose of the present study was to find

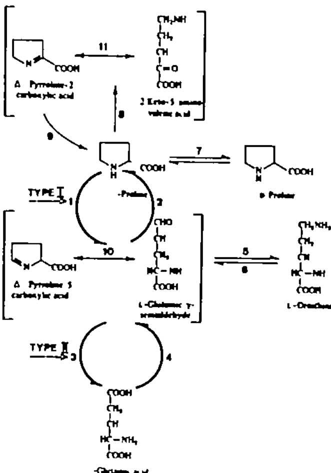


Fig 1 Metabolic reaction of proline The enzymes corresponding to the numbered reactions are: (1) proline oxidase *TYPE I* block in hyperprolinaemia type I (2) Δ pyrroline 5-carboxylic acid (5PC) reductase (3) Δ pyrroline 5-carboxylic acid (5PC) dehydrogenase *TYPE II* obvious block in hyperprolinaemia type II (4) reduction of glutamate to the semialdehyde level not enzymically characterized (5) ornithine transaminase (6) ornithine ketoacid aminotransferase (7) proline racemace (8) I amino acid oxidase (9) Δ pyrroline-2-carboxylic acid reductase

out whether diets containing little or almost no proline would lower the plasma proline levels and the urinary excretion of proline and 5PC and improve the clinical condition of two patients with hyperprolinaemia type II

CASE REPORTS

Case I Proband

Hyperprolinaemia type II was diagnosed at the age of 6 months after convulsions and encephalitis-like periods Since it was apparent that the eldest son of the family could have had the same metabolic disease which might have led to the progressive mental retardation and death of the boy (25) It was necessary to reduce the proline intake of this boy The boy was started at the age of 6 months on a diet with low proline content (*Diet I*).

While on this diet the boy s growth in height (Fig 2) and weight gain (Fig 3) were satisfactory Psychomotorically he developed normally until in February 1968 at the age of 21 months when during an infection of the respiratory tract convulsions began to recur The clinical picture was again reminiscent of acute encephalitis Complete recovery took 3 weeks (25)

After this second encephalitis-like period the dietary prescriptions were intensified by restricting the amount of proline (*Diet II*) The boy developed normally both in growth (see Figs 2 and 3) and psychomotorically

During the dietary treatment the plasma proline level and the urinary excretion of free proline (31) 5PC (25), free hydroxyproline (15) and glycine (3) were measured repeatedly every half year More accurate assays of plasma and urinary amino acids during the diet were performed by column chromatography (Beckman Unichrom) using the standard method described in the manual of the apparatus A more accurate survey of the developmental level was made after 1 year 9 months from the introduction of the restricted diet EEG was taken at 6-month intervals during the dietary treatment

Case 2 Hyperprolinaemic brother

Hyperprolinaemia type II was diagnosed at the age of 7 years Since he had not had convulsions a restricted intake of proline was not recommended although the EEG showed distinct abnormalities (25) At the age of 9 years a very low proline diet (*Diet III*) was prescribed for the boy and the excretion of proline 5PC hydroxyproline and glycine was measured daily More accurate assays of plasma and urinary amino acids were performed during the normal diet and after 18 days of the low-proline diet by column chromatography EEG was taken both before and after the dietary treatment

During the 18 days on the diet the boy was well but then he refused to continue with it and it had to be given up After this he ate normally for his age i.e approximately 70 g of protein per day and 3.5 g of proline per day (125 mg/kg per day)

DIETS

Diet I contained fruits (apple orange banana pear) and vegetables (potatoes carrots and beets) berries, a little fish and bread and Nutramigen® instead of milk This diet contained 24 g of protein and 1 5 g of proline per day Its caloric content was 1100–1200 kcal per day

Diet II was as Diet I except that the amount of Nutramigen® was lower containing an average of 19 g of protein and 1 2 g of proline per day and its caloric content was 1200–1300 kcal per day

Diet III This very low proline diet was designed to be essentially proline-free (Table 1) A synthetic mixture of amino acids containing the daily minimum requirements was used as the principal nitrogen source (21). The amino

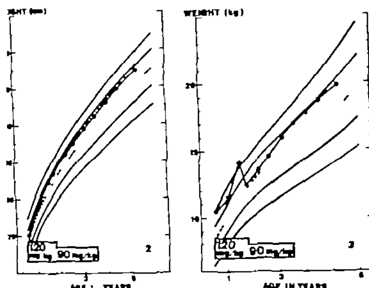


Fig. 2. The growth in height of the proband with hyperprolinaemia type II compared with that of normal Finnish children (6). The proband received 120 and 90 mg/kg of proline per day.

Fig. 3. The growth in weight of the proband with hyperprolinaemia type II compared with that of normal Finnish children (6). The proband received 120 and 90 mg/kg of proline per day.

acid mixture was made up daily and homogenized with 500 ml of orange juice and 15 g of sugar. Equal aliquots were given at 4 daily feedings. The mixture of amino acids and orange juice, equivalent to 6.4 g of protein, was supplemented with fruits, vegetables and sugar containing 5.5 g of protein, to provide total of 12 g of protein per day and approximately 900 kcal per day. The total diet was essentially proline-free except for an average daily amount of 300 mg (11 mg/kg per day), which was calculated to be the proline content of the fruits and vegetables.

RESULTS

Case I

Repeated assays showed the plasma proline level to be high (on an average 39.8 mg/100 ml) and the urinary excretion of free proline, 5PC, hydroxyproline and glycine to be elevated (347, 163, 34 and 179 mg/24 h respectively). During the reduced proline diet (Diet I) the plasma proline level declined slightly (Table 2). During the even more reduced proline diet (Diet II) following the encephalitis-like period the decline of the plasma proline level was greater. The reduction in the urinary excretion of proline and 5PC was similar. The slightly elevated plasma level of ornithine also declined, but those of other amino acids showed no significant changes (Table 3).

After the encephalitis-like period the psychomotoric development of the boy was normal. At the end of the dietary treatment, when the boy was 3 $^1/_2$ years old, evaluation of the intellectual level by the Merrill Palmer's test indicated an IQ of 90 and no signs of organic damage could be detected.

Repeated EEG examinations during the dietary treatment continued to show the slight abnormalities verified earlier. Furthermore, paroxysmal features now appeared constantly during photostimulation, suggesting an ab-

Table 1. *Essential amino acid and protein content of the very low-proline, low-protein diet*

Essential amino acids	Requirement of adults (g/day) (21)	Received by Case 2 (g/day)
L-phenylalanine	1 10	1 10
L-methionine	1 10	1 10
L-leucine	1 10	1 10
L-valine	0.80	0.80
L-lysine	0 80	0 80
L-isoleucine	0 70	0 70
L-threonine	0.50	0.50
L-tryptophan	0 25	0 25
Protein in basal diet (fruits and vegetables, containing approximately 300 mg of protein)		5.5
		12

Table 2 *Plasma proline and the urinary excretion of proline and 5PC during different dietary regimens in Case 1*

	Intake of proline		Plasma proline (mg/100 ml)	Urinary excretion of	
	mg/day	mg/kg		proline (mg/24 h)	5PC (mg/24 h)
Normal proline intake	2000	200	39.8	347 (134–1090)	163 (117–225)
Diet I	1500	170	24.1	57	111
Diet II	1200	90	9.8	20	42
Normal proline intake	100	1.5	39.0	1390	[illegible]11

mean (range)

normal sensitivity to light. After 1 1/2 years of the dietary treatment the EEG finding was distinctly pathologic, accentuated on the right side with more numerous slow spike waves. Photostimulation also brought about a foto-myoclonic response.

Case 2

Repeated assays of plasma proline during the normal diet showed the plasma level of proline to be high, on an average 38.4 mg/100 ml (Table 4) and the plasma ornithine to be also slightly elevated (See Table 3). The urinary excretion of free proline, 5PC, hydroxyproline and glycine was elevated (approximately 1800, 600, 112 and 290 mg per 24 h, respectively). During the very low proline diet (Diet III) the plasma level of proline declined rapidly to 8.1–12.2 mg/100 ml. The urinary excretion of proline was normalized, and the excretion of 5PC decreased, though it did not disappear completely (see Table 3). EEG taken on the last day of the dietary treatment showed ab-

Table 3 *Plasma amino acids (mg/100 ml) on various dietary periods in two patients with hyperprolinaemia type II*

	Normal prepubertal children (23)		Case 1		Case 2	
	Mean	Range	Diet I (170 mg/kg per day)	Diet II (90 mg/kg per day)	Normal proline intake (1.5 mg/kg per day)	Diet III (11 mg/kg per day)
Alanine	2.08	1.22–[illegible].71	2.10	1.87	1.50	[illegible]19
Arginine	0.92	0.40–1.49	0.56	0.42	0.89	0.59
Aspartic acid	0.13	0.05–0.27	0.09	0.06	0.05	0.08
Citrulline	0.37	0.[illegible]1–0.5	0.33	0.76	0.59	0.47
Halfcystine	0.73	0.54–0.92	0.65	0.46	0.54	0.40
Glutamic acid	1.62	0.34–3.68	0.75	1.40	0.99	0.60
Glycine	1.23	0.88–1.67	1.37	1.50	1.40	1.63
Histidine	0.85	0.37–1.31	0.68	0.51	0.94	0.65
Isoleucine	0.56	0.36–1.10	0.42	0.43	0.37	0.39
Leucine	1.11	0.73–2.33	0.80	0.68	0.71	0.63
Lysine	1.46	1.03–2.70	0.95	0.88	1.16	0.86
Methionine	0.21	0.16–0.23	0.17	0.[illegible]6	0.16	0.[illegible]7
Ornithine	0.44	0.36–1.13	1.53	1[illegible]	1.69	1.15
Phenylalanine	0.70	0.42–1.01	0.53	0.51	0.53	0.50
Serine	0.99	0.82–1.18	0.96	1.[illegible]0	0.88	0.93
Taurine	1.00	0.71–1.41	0.78	0.98	1.03	0.78
Threonine	0.91	0.50–1.13	0.74	0.93	1.20	1.03
Tyrosine	0.78	0.56–1.29	0.57	0.60	0.62	0.44
Valine	1.90	1.49–3.31	1.47	1.01	1.02	0.99

able 4 Plasma proline and the urinary excretion of proline and 5PC during different dietary egimens in Case 2 age 9 years weight 28 kg

	Intake of proline		Plasma proline	Urinary excretion of	
	mg/day	mg/kg	(mg/100 ml)	proline (mg/24 h)	5PC (mg/24 h)
ormal proline intake	3 500	125	38.4 (33 4–43 7)	1819 (1 47– 200)	607 (430–860)
ery low proline ntake (Diet III)					
day 1	300	11	28 9	877	588
day 3			27.6	770	531
day 5			50	51	429
day 7			19 1	135	260
day 11			18 3	178	158
day 13			1	18	57
day 16			10 0	13	68
day 18			8 1	1	27
Normal proline intake					
day 1	3 500	1 5	14	629	36
day 3			19 6	1308	475
day 8			4 0	2510	497
day 1			30 1	430	520
day 15			34 2	1340	576

mean (range).

normalities similar to those noted in numerous examinations prior to the dietary treatment

During the normal proline intake following the 18 days on very low proline diet the plasma proline values reached a hyperprolinaemic level in 12 days The urinary excretion of proline and 5PC increased rapidly in only 3 days, reaching the level of a normal diet

DISCUSSION

In our Case 2 with hyperprolinaemia type II a restriction of the dietary proline effected a slow fall in the plasma proline level and in the urinary excretion of proline and 5PC A normal plasma proline level was not reached during the 18 days dietary restriction of proline The urinary excretion of 5PC decreased but remained at the level of 27–57 mg/24 h. Such values of the urinary excretion of 5PC are elevated for 5PC cannot be found in the urine of normal children not even after the infusion of large (7 mmol/kg) intravenous proline loads (27) In our Case 1 the reduction of the dietary proline resulted in a very slow decrease of the plasma proline level and the urinary excretion of 5PC and proline These changes were related to the proline intake

In an infant with hyperprolinaemia type I described by Harries et al the restriction of dietary proline to 6 mg/kg at the age of 9 months resulted in a prompt normalization of the plasma proline level within only 2 days (12) The low-proline diet with the intake of proline (120 mg/kg per day) adjusted so as maintain the plasma level between 8 and 10 mg/100 ml was continued A similar response was noted in a 4-month-old female infant with hyperprolinaemia type I by Newns (18) and in a 13-year-old girl with hyperprolinaemia type I and renal insufficiency by Goyer et al (11)

The fact that a dietary restriction may result in a fall of the plasma proline level even down to normal seems to suggest that endogenous synthesis (21) is not a very important source of circulating proline Harries et al put forward an opinion based on the appearance of a rash in their patient 4 weeks after starting the low-proline diet According

Tablc 2 *Plasma prolinc and the urinary excretion of proline and 5PC during different dietar regimens in Case 1*

	Intake of proline		Plasma proline (mg/100 ml)	Urinary excretion of	
	mg/day	mg/kg		proline (mg/24 h)	5PC (mg/24 h)
Normal proline intake	000	200	39 8	347 (134–1090)	163 (117–_5)
Diet I	1500	170	4 1	57	111
Diet II	1700	90	9 8	70	4
Normal proline intake	2100	1_5	39 0	1390	211

mean (range)

normal sensitivity to light After 1 1/2 years of the dietary treatment the EEG finding was distinctly pathologic accentuated on the right side with more numerous slow spike waves Photostimulation also brought about a foto myoclonic response

Case 2

Repeated assays of plasma prolinc during the normal diet showed the plasma level of pro line to be high on an average 38 4 mg/100 ml (Table 4) and the plasma ornithine to be also slightly elevated (See Table 3) The urinary excretion of free proline 5PC hydroxyproline and glycine was elevated (approximately 1800 600 112 and 290 mg per 24 h respectively) During the very low proline diet (Diet III the plasma level of proline declined rapidly to 8 1–12 2 mg/100 ml The urinary excretion of proline was normalized and the excretion of 5PC decreased though it did not disappear completely (see Table 3) EEG taken on the last day of the dietary treatment showed ab

Table 3 *Plasma amino acids (mg/100 ml) on various dietary periods in two patients with hyper prolinaemia type II*

	Normal prepubertal children (_3)		Case 1		Case	
			Diet I (170 mg/kg per day)	Diet II (90 mg/kg per day)	Normal pro-line intake (125 mg/kg per day)	Diet III (11 mg/kg per day)
	Mean	Range				
Alanine	2 08	1 2_– 71	10	1 87	1 50	19
Arginine	0 9	0.40–1 49	0 56	0 4	0 89	0 59
Aspartic acid	0 13	0 05–0 27	0 09	0 06	0 05	0 08
Citrulline	0 37	0 1–0 5	0 33	0 6	0 59	0 47
Halfcystine	0 73	0 54–0 92	0.65	0 46	0 54	0 40
Glutamic acid	1 6_	0 34–3 68	0 75	1 40	0 99	0 60
Glycine	1 23	0 88–1 67	1 37	1 50	1 40	1 63
Histidine	0 85	0 37–1 31	0 68	0 51	0 94	0 65
Isoleucine	0 56	0 36–1 10	0 42	0 43	0 37	0 39
Leucine	1 11	0 73–2 33	0 80	0 68	0 71	0 63
Lycine	1 46	1 03–2.70	0 95	0 88	1 16	0 86
Methionine	0 21	0.16–0 _3	0 17	0 26	0 16	0 27
Ornithine	0 44	0.36–1 13	1 53	1 22	1 69	1 15
Phenylalanine	0 70	0 4 –1 01	0.53	0 51	0 53	0.50
Serine	0 99	0 82–1 18	0 96	1.20	0 88	0.93
Taurine	1 00	0 71–1 41	0 78	0 98	1 03	0 78
Threonine	0 91	0.50–1 13	0 74	0 93	1 70	1 03
Tyrosine	0 78	0.56–1 79	0.57	0 60	0 6	0.44
Valine	1 90	1 49–3 31	1 47	1 01	1 02	0 99

Table 4 *Plasma proline and the urinary excretion of proline and 5PC during different dietary regimens in Case 2 age 9 years weight 28 kg*

	Intake of proline		Plasma proline	Urinary excretion of	
	mg/day	mg/kg	(mg/100 ml)	proline (mg/24 h)	5PC (mg/24 h)
Normal proline intake	3 500	125	38 4 (33 4–43 7)	1819 (1247–2200)	*607 (430–860)
Very low proline intake (Diet III)					
day 1	300	11	28 9	877	588
day 3			27 6	770	531
day 5			25 0	51	479
day 7			19 1	135	260
day 11			18 3	178	158
day 13			1	18	57
day 16			10 0	13	68
day 18			8 1	12	77
Normal proline intake					
day 1	3 500	125	14	629	362
day 3			19 6	1308	475
day 8			4 0	510	497
day 1			30 1	430	520
day 15			34 2	1340	576

* mean (range)

normalities similar to those noted in numerous examinations prior to the dietary treatment

During the normal proline intake following the 18 days on very low proline diet the plasma proline values reached a hyperprolinaemic level in 12 days The urinary excretion of proline and 5PC increased rapidly in only 3 days reaching the level of a normal diet

DISCUSSION

In our Case 2 with hyperprolinaemia type II a restriction of the dietary proline effected a slow fall in the plasma proline level and in the urinary excretion of proline and 5PC A normal plasma proline level was not reached during the 18 days dietary restriction of proline The urinary excretion of 5PC decreased but remained at the level of 27–57 mg/24 h Such values of the urinary excretion of 5PC are elevated for 5PC cannot be found in the urine of normal children not even after the infusion of large (2 mmol/kg) intravenous proline loads (27) In our Case 1 the reduction of the dietary proline resulted in a very slow decrease of the plasma proline level and the urinary excretion of 5PC and proline These changes were related to the proline intake

In an infant with hyperprolinaemia type I described by Harries et al the restriction of dietary proline to 6 mg/kg at the age of 9 months resulted in a prompt normalization of the plasma proline level within only 2 days (12) The low proline diet with the intake of proline (120 mg/kg per day) adjusted so as maintain the plasma level between 8 and 10 mg/100 ml was continued A similar response was noted in a 4-month-old female infant with hyperprolinaemia type I by Newns (18) and in a 13-year-old girl with hyperprolinaemia type I and renal insufficiency by Goyer et al (11)

The fact that a dietary restriction may result in a fall of the plasma proline level even down to normal seems to suggest that endogenous synthesis (21) is not a very important source of circulating proline Harries et al put forward an opinion based on the appearance of a rash in their patient 4 weeks after starting the low proline diet According

Table 5 *Urinary excretion of proline and 5PC compared with the dietary intake of proline in patients with hyperprolinaemia type II*

Patient	Age (years)	Proline intake (mg/24 h)	Plasma proline (mg/100 ml)	Urinary excretion of proline (mg/24 h)	Urinary excretion of 5PC (mg/24 h)	Recovery (%)
Case 1	4	2100	39 0	1390	211	76
Case 2	9	3500	38 4	1819	607	69

to them endogenous proline synthesis was not sufficient to meet the requirement (12) This is in agreement with the findings of Jürgens & Dolif in man (14) and those of Adkins and co workers in young rats (2) They suggest that proline might thus be regarded as semi essential

Efron showed in a study of a patient with hyperprolinaemia type I that the activity of proline oxidase in a post mortem liver was present though reduced (8) Harries et al showed that the recovery of urinary proline in their patient and in some of the patients reported to have hyperprolinaemia type I was very small only about 7% (12) The metabolic block in hyperprolinaemia type II has not yet been verified The presence of 5PC in the urine suggests a defect in the conversion of 5PC into glutamic acid This hypothesis is also supported by the increased excretion of 5PC during a loading with ornithine in our patients (26) The assumption that the increase in the urinary excretion of 5PC during ornithine infusion may originate from the infused ornithine is in accordance with what is known of the kinetic constant of ornithine ketoacid aminotransferase for L-ornithine the equilibrium tends strongly towards 5PC (29)

Table 5 shows the recovery of urinary proline and 5PC in our patients with hyperprolinaemia type II In performing these calculations no allowance has been made for faecal losses of protein and proline endogenous synthesis of proline the conversion of proline into hydroxyproline of collagen with its subsequent degradation the conversion of 5PC into ornithine nor any other possible metabolic reactions of proline and 5PC perhaps still unknown The percentage recovery in our patients was great 76 and 69% This suggests that the metabolic block in the conversion of 5PC into glutamic acid is almost complete and that the possible alternative metabolic pathways do not have any significance in the degradation of proline in man (Fig 1) In this respect hyperprolinaemia type II resembles classical phenylketonuria (5) This difference in the completeness of the metabolic block between type I and type II hyperprolinaemia may perhaps explain the differences of the dietary treatment

5PC is a no threshold metabolite and it cannot be determined in the blood plasma even in patients with hyperprolinaemia type II (26) I have succeeded in measuring it from blood plasma of our hyperprolinaemia type II patients during the infusion of large (2 mmol/kg) intravenous ornithine loads (26). The slightly increased plasma level of ornithine during normal proline intake in our second patient with type II also suggests a defect in the conversion of 5PC into glutamic acid

The rate of urinary excretion of 5PC in our patients is high This suggests that the activity of 5PC dehydrogenase in man can be higher than what Strecker observed it to be in the ox liver about 0 2 μmol/min per mg of protein (30)

The 5PC which has been excreted in the urine of patients with hyperprolinaemia type II and has reacted with *o* aminobenzaldehyde (*o* ABA) can be heterogenous (4 24) It is

possible that other oxidative reactions of proline and probably also of 5PC may exist in man One study (7) indicated the presence of an enzyme oxidizing L-proline into Δ^1-pyrroline ?-carboxylate in the rat kidney This reaction has not been established as significant in animal metabolism (1) But can this reaction, whether oxidative or not be significant in patients with hyperprolinaemia type II?

In the present study low-proline diet had no clear effect on the electroencephalographic abnormalities This is contrary to the observations of Harries et al on their infants with *hyperprolinaemia type I* The mental development and growth of our proband during the period of the low-proline diet were satisfactory and no encephalitis-like periods occurred

The relation between the metabolic defect and the clinical abnormalities in hyperprolinaemia type II is not entirely clear All the patients with hyperprolinaemia type II described have had some findings EEG abnormalities *if nothing else suggestive of mild* or minimal brain damage unlike the patients with hyperprolinaemia type I (10 17) and hydroxyprolinaemia (19) some of whom have clinical findings not even EEG abnormalities I assume that the elevated intracellular levels of proline and 5PC or metabolites in hyperprolinaemia type II do not in themselves cause brain damage though they help to make the brain more sensitive to such damage This sensitivity might manifest itself as EEG abnormalities The brain damage is caused by some other factors Before the encephalitis-like periods both our Case I and the patient of Selkoe had fever and other manifestations of infection Infection might constitute the factor causing the permanent or partial reversible damage to the brain rendered more sensitive by the metabolic defect in *hyperprolinaemia* type II The sensitivity of the brain to the damage can perhaps be reduced by means of a low proline diet

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CLINICAL APPLICATIONS OF ECHOCARDIOGRAPHY IN INFANTS AND CHILDREN

III *Estimation of Left and Right Ventricular Size a Comparison between Echocardiography and Angiocardiography*

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ABSTRACT Lundström, N -R. and Mortensson, W (Departments of Pediatrics and Radiology University of Lund, Sweden). Clinical applications of echocardiography in infants and children. III. Estimation of left and right ventricular sizes comparison between echocardiography and angiocardiography Acta Paediat Scand, 63:257 1974 —Intracardiac distances can be measured by echocardiography which is a non-invasive technique. The aim of the present study was to investigate if the size of the left and right ventricle could be estimated by echocardiography This was accomplished by a comparison with angiocardiography The left ventricular internal dimension measured by echocardiography was compared with the long axis of the left ventricle measured on an angiocardiogram in 60 patients. A comparison between a right ventricular dimension measured by echocardiography and the size of the right ventricle evaluated from an angiocardiogram was made in 84 patients. A fairly good positive correlation was found between the left ventricular internal dimension measured by echocardiography and the long axis of the left ventricle measured on the angiocardiogram (r=0.93). As regards right ventricular size, it was found that echocardiography could separate a right ventricle of normal size from clearly enlarged right ventricle. The conclusion of this investigation is that the echocardiographic examination can serve as a useful, non-invasive, semiquantitative method for the evaluation of left and right ventricular sizes in infants and children.

KEY WORDS: Echocardiography ultrasonics/diagnostic use, left ventricle right ventricle, infants, children

Information concerning the size of the ventricles is of course valuable in the diagnostic investigation of a patient with heart disease This information is most easily obtained by a plain chest roentgenogram There are however considerable limits to this technique For a more detailed analysis actual measurements of the ventricular volume must be performed For the left ventricle this is achieved by means of angiocardiography (1 6 16) or by an indicator dilution technique (36) and for the right ventricle by angiocardiography (19 4) These investigations however do require heart catheterization In the last few years echocardiography has been used as a non-invasive method to measure the left ventricular size (3 8 12, 13 14 30 31 32 33) and the right ventricular size (5) in adults. The same technique has been used in infants and children (25 27 37 39) but a comparison with other techniques available for measuring ventricular size seems not to have been reported earlier

In a previous report from this laboratory it was shown that information about left and right ventricular size could be obtained in in-

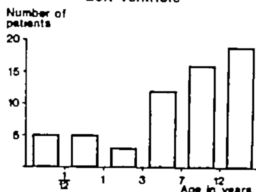


Fig 1 Age distribution of the material where a comparison was made between a left ventricular internal dimension measured by echocardiography and the length of the left ventricle measured on an angiocardiogram

fants and children without heart disease by echocardiography (25) and a test of the reproducibility of the echocardiographic measurements was also reported The pupose of the present investigation was to evaluate the use of echocardiography for estimating the size of the left and right ventricles by comparison with the results obtained at angiocardiography

MATERIAL

The present investigation started as part of a general comparison between echocardiography and angiocardiography During a period of 18 months every infant and child examined by angiocardiography also had an echocardiographic examination In this material 65 infants and children had a satisfactory angiocardiogram of the *left ventricle* Out of these 65 patients 5 had to be excluded One of these patients 3 months old with a ventricular septal defect had a severe obstructive bronchitis with hyperinflation of the lungs. It was impossible to get any echoes at all from the heart in this patient, presumably because there was lung tissue in front of the heart Two patients had to be excluded due to abnormal positioning of the heart situs inversus in one and a mediastinal tumour dislocating the heart in the other The remaining two patients both had a corrected transposition of the great arteries with ventricular inversion (levo-transposition) In this malformation the spatial orientation of the interventricular septum is abnormal. Since the echocardiographic estimation of left ventricular size is based on a normal relation between the interventricular septum and the posterior heart wall, these patients had to be excluded

Ninety-two patients had a satisfactory angiocardiogram of the *right ventricle* From this material 8 patients had to be excluded Three of them were those referred to above where no cardiac echoes could be obtained or where the heart was abnormally placed. Three patients had a corrected transposition of the great arteries with ventricular inversion and were excluded for reasons referred to earlier In the two remaining patients it was impossible to obtain any echoes at all from the interventricular septum These two infants died and at autopsy it was found that they both had a single ventricle

The age distribution of the patients in whom a comparison between echocardiography and angiocardiography was made is given in Figs 1 and 2 All patients had sinus rhythm

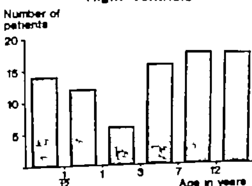


Fig 2 Age distribution of the material where a comparison was made between the size of the right ventricle estimated by echocardiography and angiocardiography

METHODS

Echocardiographic examination

Generally the echocardiographic examination was performed the day before the angiocardiographic investigation No premedication was given to these patients Some of the younger children were so nervous that satisfactory echocardiographic registrations could not be obtained These children were re-examined some hours after the angiocardiographic investigation while still sedated by the premedication given for the angiocardiography In this way satisfactory echocardiographic registrations could be made in these nervous children The patients were examined in the supine position during normal respiration

The principles of echocardiography using pulsed, reflected ultrasound are well described earlier (7) A commercially available ultrasonoscope (Smith Kline Eskoline 20) was used The repetition rate of this instrument is 1000 pulses/sec A 2 25 MHz transducer with a diameter of 1 9 cm was used A water-soluble gel was used to obtain airless contact between the transducer and the skin The registrations were made on Polaroid film and an electrocardiogram was recorded simultaneously as a reference. A detailed description about the technique used in this investigation for echocardiographic examinations of the interventricular septum and the posterior heart wall has been described earlier (25) and will not be reported here A short outline will however be presented The transducer was placed in the fourth left intercostal space at the sternal border The echo from the anterior mitral leaflet with its characteristic movement was identified first From this position the transducer was angulated slightly in

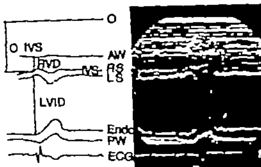


Fig 3 Echocardiogram of the interventricular septum and the posterior left ventricular wall. The top of the figure represents the anterior direction. The sites where measurements were made of left ventricular internal dimension (*LVID*), right ventricular dimension (*RVD*) and of the distance between the anterior chest wall and the right side of the interventricular septum (*0-IVS*) are indicated in the line drawing. Abbreviations: *0*=anterior chest wall. *AW*=anterior wall of the right ventricle. *RS*=right side of interventricular septum (*IVS*). *LS*=left side of interventricular septum. *Endo*=endocardial posterior wall of left ventricle. *PW*=posterior epicardial wall of left ventricle. *ECG* electrocardiogram.

the lateral and caudal direction. A transducer position was thereby *found where the echoes necessary* for the intended echocardiographic measurements could be obtained *simultaneously*. These echoes origin from the posterior left ventricular wall and its endocardial border the interventricular septum (two parallel echoes) and the anterior heart wall (Fig 3). This technique was originally presented by Popp et al (32), and further details about its *application in order to standardize* the method have been presented by several authors (10 26, 31 33). Two circumstances were considered important in order to make the measurements reproducible between different subjects (1) *transducer position* in the fourth *intercostal space at the left sternal border* and *(2) transducer direction* slightly lateral and inferior *to the direction* where the characteristic echo from the anterior mitral leaflet is obtained. With the transducer placed in this way uncharacteristic echoes can often be seen between the echoes from the endocardial posterior left ventricular wall and from the left side of the interventricular septum. These echoes *presumably originate* from the chordae tendineae (10) or from the posterior mitral valve (10 26) and indicate that the direction is close to the mitral valve. The reproducibility of the echocardiographic measurement using this technique has been reported earlier (25).

The following measurements were made: the distance between the endocardial echo from the *posterior* left ventricular wall and the echo from the left side of the interventricular septum (*left ventricular internal dimension*, LVID Fig 3), the distance between the echo from the right side of the interventricular septum and the posterior border of the dense echoes from the *anterior chest* wall (right ventricular dimension RVD Fig. 3), and the distance between the echo from the right side of the interventricular *septum and* the echo from the surface of the anterior chest wall (0-IVS, Fig. 3). All these measurements were made *at end-diastole defined as the peak of* the R-wave in the simultaneously recorded electrocardiogram. The anterior margin of the echofree space anterior to the right *side of the interventricular* septum (Fig 3) was not always distinct. The measurement of the right ventricular dimension (RVD) was therefore taken as the *maximal* distance between the echo from the anterior (right) side of the interventricular septum and the posterior *margin of the dense* echoes from the anterior chest wall. For comparison with angiocardiography *means* of 5 individual measurements were used for all other distances.

Angiocardiographic examinations

The heart catheterizations and angiocardiographic examinations were performed without general anaesthesia (2). Children and infants more than 3 months of age received a premedication with a mixture of Demerol® Largactil® and Phenergan. The infants below 3 months of age received no premedication. The radiological measurements were made on full-size angiocardiograms. The requirements of the angiocardiograms used in this study were that the left or right ventricle was clearly outlined irrespective of where the contrast-injection had been made. It has earlier been shown (10 14) that the echocardiographic left ventricular dimension is an oblique dimension somewhere between the long axis and the short axis of the left ventricle. A direct comparison between similar distances was therefore not attempted. The angiocardiographic measurements were made on distances which could easily be defined and outlined. For the left ventricle the long axis between the midpoint of the aortic valve and the apex of the ventricle (Fig 4) in the lateral projection was used. For the right ventricle the long axis between the midpoint of the pulmonic valve and the apex of the ventricle was measured on an angiocardiogram in the lateral projection (*Fig 5*). *The measurements were made at end-diastole*. A correction for the magnification was not made. The focus-film distance was 100 cm.

RESULTS

The results of the measurements of the left ventricular internal dimension by echocardiography (LVID Echo) compared with the corresponding left ventricular long axis on the angiocardiogram (LVL Angio) are shown in Fig. 6. There is a fairly good correlation over a wide range of values.

A comparison between the echocardiographic *measurement of the distance between the an*terior chest wall and the right side of the inter

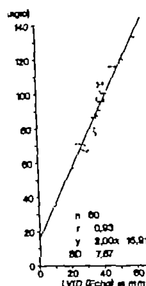


Fig 4 Angiocardiogram of the left ventricle in lateral projection The site where the measurement of the left ventricular long axis was made is indicated



Fig 5 Angiocardiogram of the right ventricle in lateral projection The site where the measurement of the right ventricular long axis was made is indicated

ventricular septum (0-IVS Echo) and the corresponding right ventricular long axis on the angiocardiogram (RVL Angio) is shown in Fig 7 There is a positive correlation but with a rather large scatter Since the estimation of right ventricular size in this way seemed unsatisfactory another approach was tried All the angiocardiograms of the right ventricle were examined by one of the authors and placed into one of three groups (1) clearly enlarged right ventricle (2) slightly or questionably enlarged right ventricle or (3) a right ventricle of normal size The other author made all the corresponding echocardiographic measurements of the distance between the anterior chest wall and the right side of the interventricular septum (0-IVS) and of the right ventricular dimension (RVD) All values lying within the 95% prediction interval of normal in relation to the body weight (25) were classified as normal All values above the upper 95% prediction limit were considered indicative of an enlarged right ventricle The results of these independent estimations were compared and are presented in Figs 8 and 9 As can be seen (Fig 8) there is once again an unsatisfactory overlapping between normal and enlarged conditions when the right ventricular enlargement is based echocardiographically on the distance 0-IVS The right ventricular dimension (RVD) measured by echocardiography can however separate the patients with a clearly enlarged right ventricle from those with a right ventricle of normal size (Fig 9) The RVD-values on echocardiography in the group with a clearly enlarged right ventricle on angiocardiography were between 11 and 23 mm (mean 15 0 mm) The group classified as having a slightly en-



g 6 A comparison between the measurements of the ft ventricular internal dimension made by echocardiography (*LVID Echo*) and the left ventricular long axis measured on the angiocardiogram (*LVL Angio*) The regression line is indicated

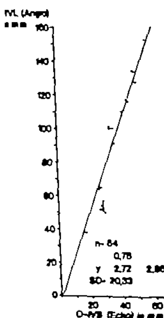


Fig 7 A comparison between the measurements of the distance between the anterior chest wall and the anterior (right) side of the interventricular septum measured by echocardiography (*D-IVS Echo*) and the right ventricular long axis measured on the angiocardiogram (*RVL Angio*) The regression line is indicated

RV size Angio	RV size Echo (D IVS) Enlarged	Normal
Enlarged	37	16
Slightly enlarged	3	11
Normal	2	16

Fig 8 A comparison between the radiological estimation of right ventricular size (normal, slightly enlarged or enlarged) based on the angiocardiogram and the echocardiographic estimation of right ventricular size (normal or enlarged) based on the distance between the anterior chest wall and the anterior (right) side of the inter ventricular septum.

larged right ventricle on angiocardiography had corresponding RVD-values of 11–12 mm (mean 11 4 mm) This difference indicates that the RVD-values increase with increasing rela tive size of the right ventricle

DISCUSSION

Two factors seems to be of primary importance in a discussion about the use of echoecardiography for the determination of ventricular size identification of the intracardiac echoes and standardization of the technique It has been shown that rapid injection of indiocya nine green into a herart cavity produces dense ultrasound echoes (20) The echoes from the anterior heart wall the interventricular septum and the posterior wall of the left ventricle have

RV size Angio	RV size Echo (RVD) Enlarged	Normal
Enlarged	52	
Slightly enlarged	8	6
Normal		16

Fig 9 A comparison between the radiological estimation of right ventricular size (normal, slightly enlarged or enlarged) based on the angiocardiogram and the echocardiographical estimation of right ventricular size (normal or enlarged) based on the right ventricular dimension (*RVD*).



Fig 4 Angiocardiogram of the left ventricle in lateral projection The site where the measurement of the left ventricular long axis was made is indicated



Fig 5 Angiocardiogram of the right ventricle in lateral projection The site where the measurement of the right ventricular long axis was made is indicated

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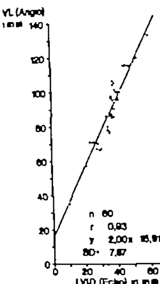


Fig 6 A comparison between the measurements of the left ventricular internal dimension made by echocardiography (*LVID Echo*) and the left ventricular long axis measured on the angiocardiogram (*LVL Angio*) The regression line is indicated.

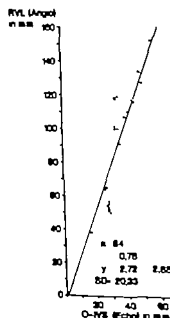


Fig 7 A comparison between the measurements of the distance between the anterior chest wall and the anterior (*right*) side of the interventricular septum measured by echocardiography (*O-IVS Echo*) and the right ventricular long axis measured on the angiocardiogram (*RVL Angio*) The regression line is indicated.

RV size Angio	RV-size Echo (O IVS) Enlarged	Normal
Enlarged	37	18
Slightly enlarged	3	11
Normal	2	18

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Fig 9 A comparison between the radiological estimation of right ventricular size (normal slightly enlarged or enlarged) based on the angiocardiogram and the echocardiographical estimation of right ventricular size (normal or enlarged) based on the right ventricular dimension (*RVD*).

all been identified by the use of this method (9 20) A standardization of the technique has been discussed by several authors (10 26 30 31 33) The importance of the direction of the ultrasonic beam being close to the anterior mitral leaflet has been stressed If the transducer is directed more in the caudal direction it is possible that the echo from the posterior left ventricular wall will be difficult to distinguish from an echo from the posterior papillary muscle An ultrasound echo is only obtained when the ultrasonic beam is directed perpendicular to the echogiving structure By requiring simultaneous echoes from the interventricular septum and the posterior left ventricular wall the direction is further limited to those parts where the interventricular septum and the posterior heart wall are parallel to each other (10 14) The standardization of the technique seems to be reasonably good this is reflected in the reproducibility of these measurements (25)

Estimation of left ventricular size

In adults echocardiographic estimations of the left ventricular volume based on distance measurements have been compared with left ventricular volume measurements based on angiocardiography (12 13 14 30 31) or on the Fick principle (33) In some of these reports a comparison was also made between the echocardiographic measurement of a left ventricular internal dimension and some angiocardiographic measurements of a left ventricular distance (13 14 30 31) Fairly good positive correlations were reported (r values varying between 0 60 and 0 90) A direct comparison with the results of the present investigation is however not possible since the angiocardiographic measurements have been made by quite different techniques The fairly good positive correlation in the present study between echocardiographic and angiocardiographic distance measurements of the left ventricle in infants and children does indicate that the echocardiographic measurements can be used as a semiquantitative measurement of left ventricular size

The main limitation in actual volume estimation based on echocardiography is that only one dimension is measured (10) Variations in the shape of the left ventricle and asynergy in left ventricular wall motion can therefore in some cases make actual echocardiographic measurements of left ventricular volumes difficult (34 38) Despite these limitations direct comparisons between echocardiographic and angiocardiographic estimations of left ventricular volumes have shown rather good correlations in adults (12 13 14 30 31) The aim of the present investigation was however not to measure volumes but to compare distance measurements as indirect signs of left ventricular size

The value of estimating left ventricular size by angiocardiography has been assessed by several authors in various forms of heart disease in infants and children e g in left right shunt distal to the atrioventricular valves before (22 29) or after surgical treatment (23) in the evaluation of patients with shunts at several sites (17) in primary myocardial disease (15) or in total anomalous pulmonary venous return (18) The information about the left ventricular size should also of course be of value in the diagnosis of the group of malformations commonly referred to as the hypoplastic left heart syndrome (27) The echocardiographic estimation of left ventricular size in the way it is used in the present investigation cannot quantify the volume of the left ventricle The results indicate however that the method can provide semiquantitative information about the left ventricular size This could be a useful information in the first examination of a patient or in the follow-up of some patients since the method is non invasive and easy to repeat

Estimation of right ventricular size

With the technique used in the present investigation to obtain echoes from the interventricular septum it is evident that the echofree

space representing the right ventricle reflects only a small part of the right ventricle (Fig 3) In an earlier investigation (25) it was shown that the right ventricular dimension measured in this way (RVD) was of about the same order in all infants and children without heart disease irrespective of age weight or height In the case of a dilated right ventricle it is evident that the ultrasonic beam passes through a larger part of the right ventricle It has been pointed out that the position of the patient influences the echocardiographic right ventricular dimension (11) Rolling the patient to his left increases this dimension (11) In order to obtain reproducible measurements it is therefore essential that the patient is examined in the supine position

In the present investigation it was sometimes difficult to clearly outline the anterior border of the echofree space representing the right ventricle The same difficulty has previously been reported by others (5) Recently it has been reported that a focussed transducer (21) or a transducer with a higher frequency (5–10 MHz) can give a better resolution (35) in the near field and thereby better delineate the anterior border of the echofree space representing the right ventricle Such transducer were however not available for the present investigation

In the present investigation an attempt was made to estimate the size of the right ventricle by measuring the distance between the anterior chest wall and the right side of the interventricular septum It is however clear that this method is inferior to measurement of the right ventricular dimension (RVD) for distinguishing a normal sized right ventricle from an enlarged right ventricle

The use of echocardiography in detecting volume overload of the right ventricle has been previously described both in adults (5) and children (28 37) using the same technique as in the present investigation It was stated that a significant volume overload could be detected by echocardiography but a direct comparison with angiocardiography was not reported

From the present comparison with angiocardiography it is however evident that with the method reported here it is possible to separate a right ventricle of normal size from a right ventricle which is clearly enlarged It is also likely that the RVD will increase with increasing dilatation of the right ventricle Only a semiquantitative estimation of right ventricular size can however be made by the method used in this study This information can however be of use and presumably provide more information on the right ventricular size than an ordinary plain chest roentgenogram.

Limitations to echocardiographic estimations of left and right ventricular size

A satisfactory recording of echoes from the interventricular septum and the posterior left ventricular wall allowing estimations of left ventricular internal dimension and right ventricular dimension could be obtained in 92% and 91% of the cases for the left and right ventricle respectively In about 3% of the patients a satisfactory echocardiographic examination could not be obtained at all In the remaining 5 patients satisfactory echoes from the interventricular septum could not be found Two of these patients died and the diagnosis of a single ventricle was verified at autopsy This observation is in agreement with earlier echocardiographic findings in patients with a single ventricle (4) The other 3 patients all had congenitally corrected transposition with ventricular inversion (levo-transposition) diagnosed by angiocardiography By angiocardiography it could also be demonstrated that an interventricular septum existed in these patients The inability to demonstrate an interventricular septum by echocardiography in these patients is presumably due to the unusual spatial orientation of the interventricular septum in this malformation An ultrasound echo is only obtained when the ultrasonic beam is directed perpendicular to the echo-giving structure

Interposition of lung tissue in front of the heart making an echocardiographic examination impossible was found in only one patient Obesity was not found to be a problem in obtaining satisfactory echoes contrary to what has been found in adults (30)

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Interposition of lung tissue in front of the heart making an echocardiographic examination impossible was found in only one patient Obesity was not found to be a problem in obtaining satisfactory echoes contrary to what has been found in adults (30)

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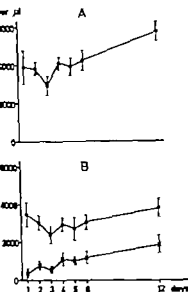


Fig 1 Average concentration of lymphocytes in the blood of 6 normal infants ± S E M Day 1 = day of delivery (*A*) ●—● All lymphocytes. (*B*) ●—● Larger lymphocytes. ×—× Small adult-type lymphocytes.

^{3}H-thymidine (spec act 1 9 Ci/mM) the other with 2.5 µCi ^{3}H-cytidine (spec act. 3 0 Ci/mM). The suspension was shaken once during the incubation period which continued for 90 min, at 37°C Thereafter the cells in the supernatant were concentrated by slight centrifugation, and smears prepared from the resuspended sediment The smears were dipped in Kodak NTB-2 emulsion and exposed for 14 days, at 4°C. In the ^{3}H-thymidine slides at least 2000 lymphocytes were counted or until 50 labelled lymphocytes had been encountered, all labelled cells showed heavy incorporation of thymidine. In the ^{3}H-cytidine slides, the grain counts over 100 successive lymphocytes were determined background activity varied from 0 to 4 grains per cell area, the grain counts given are not corrected for background.

Leukocyte counting and staining of smears were carried out by standard laboratory techniques At least 300 leukocytes were included in each differential count

RESULTS

The concentration of lymphocytes in the blood of normal babies in the neonatal period is shown in Fig 1 The temporary fall in total lymphocyte concentration during the first days after delivery has been demonstrated by Xanthou (14) Fig 1 B shows that this is due to a decline in the concentration of larger lymphocytes. Very few small lymphocytes of adult type are present at birth (average concentration 370 per µl) but during the first 2 weeks of extra-uterine life their number rises so that at the end of 2 weeks they constitute approximately 35% of the total number of lymphocytes or 1900 per µl

The concentration in the blood of cells in DNA synthesis as demonstrated by their incorporation of ^{3}H-thymidine was found to be approximately 50 per µl at birth in agreement with the findings of Winter et al (13) During the first 2 days of extra-uterine life the concentration decreased to 9 per µl blood (Fig. 2) Thereafter their concentration rose steeply the maximum concentration observed was 75 per µl on day 6 At the last blood sampling on day 12, their number had again declined. This sequence decrease during the first one or two days followed by a steep increase was seen in all infants In the blood of neonates with bacterial infections preliminary studies have revealed increased numbers of lymphocytes in DNA synthesis up to 350 per µl (2)

Almost all cells in DNA synthesis were large cells (diameter 14–20 µm in the smears) Their nuclei were ovoid or kidney shaped, characteristically placed to one side of the cell and leptochromatic They had a moderate amount of cytoplasm which was weakly basophilic often of a grey-blue shade These cells have been described as transitional cells by Yoffey et al (15) Small adult-type

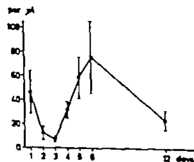


Fig 2 Average concentration of ^{3}H-thymidine-labelled cells in the blood of 6 normal infants, ±S.E.M.

Acta Pædiat Scand 63 266–270 1974

CHANGES IN BLOOD LYMPHOCYTES DURING THE NEONATAL PERIOD

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ABSTRACT Andersen V and Andersen E. (Departments of Paediatrics and Medicine Rigshospitalet, Copenhagen, Denmark) Changes in Blood Lymphocytes during the Neonatal Period Acta Paediat Scand 63 266 1973.—Blood lymphocytes were studied in 6 normal neonates during the first 2 weeks after delivery At birth the larger lymphocytes constituted 90% these cells decreased in number until day 3 and then increased again. Small, adult type lymphocytes showed a steady increase in numbers during the observation period. DNA-synthesizing cells were demonstrated by means of H-thymidine incorporation followed by autoradiography At birth, their average concentration was 47 per μl they decreased in number to 8 per μl on day 3 and rose to a maximum of 76 per μl on day 6. RNA metabolic activity was evaluated by the uptake of ³H-cytidine Similar but less pronounced findings were obtained preceding the changes in the number of DNA-synthesizing cells by one or two days. It is hypothesized that the increase from day 2 is due to activated lymphocytes and reflects the stimulation of the immune apparatus through antigenic exposure

KEY WORDS. Lymphocytes, DNA-synthesizing blood cells, newborn infants

The transfer at birth from the usually sterile environment within the uterus to ordinary contaminated life must mean a decisive challenge to the immune apparatus Antibody production has been extensively studied in infants (1 5) but few direct studies of lymphoid cells in the neonatal period have been carried out in man

In the present study blood lymphocytes were examined with respect to number morphology and incorporation of precursors of DNA and RNA Healthy infants were studied at birth and followed sequentially for 2 weeks after delivery

MATERIAL AND METHODS

Six full-term infants with uncomplicated gestation, delivery and neonatal development constitute the normal material in particular there was no evidence of infection in mother or child Blood samples were obtained immediately after delivery daily for the next 5 days and 12 days after birth.

The term lymphocytes is employed in the present study to include all non-phagocytic nucleated blood cells with the exception of cells that by morphological criteria belonged to the erythroid and myeloid series. The lymphocytes were classified in two groups, small and larger The small lymphocytes were characterized by their densely pachychromatic nucleus, with only a narrow rim of cytoplasm cell diameter was maximally 8 μm in the smears This is the type most frequently found in normal adult blood All lymphocytes not fulfilling these strict criteria were classified as larger lymphocytes the distinction between these cells and monocytes was based on the capacity to phagocytize polystyrene particles *in vitro* (see below)

As a labelled DNA precursor H-thymidine was used experiments were carried out in parallel employing H cytidine which is incorporated into both RNA and DNA Capillary blood was obtained by heel-prick. 250 μl were collected into a suspension containing 25 IU of heparin 250 μl of 6% dextran (mol wt 70000) in 0 9% NaCl 100 μl of 0 5% Latex particles (diameter 1 1 μm) and 250 μl of medium TC 199 Two such samples were obtained and one of these was incubated with 2.5 μCi

ated and the possibility that some are early myeloid cells is not excluded

The changes in DNA-synthesizing cells after birth have not been studied before We found a decrease during the first few days after delivery (Fig. 2) from day 3 to day 6, the concentration rose tenfold and then again decreased Cytidine incorporation although not a quantitative measure of RNA synthesis (8) does give an indication of RNA metabolic activity in the cell This parameter showed changes (Fig 4) parallel to those in the number of DNA-synthesizing cells and actually preceding these by one or two days an interval which is also encountered when lymphocytes are stimulated by antigen *in vivo* or *in vitro* (12)

It is not known whether the changes observed are of an immunological nature It seems probable however that they are related to the massive exposure to antigens which takes place after delivery especially in the gastrointestinal tract In germ-free piglets changes in the circulating lymphocytes are detectable within 48 hours after antigenic stimulation (11) It is known from experiments in sheep (7) that following a local antigenic stimulus lymphocytes are retained in the regional lymph nodes within 2–3 days lymphocytes with activated RNA and DNA synthesis are released from the lymph nodes and pass via the lymphatics to the blood, thereby disseminating the immune response It has been demonstrated (3) that in the foetal lamb almost all the cells of intestinal lymph are small lymphocytes, and only a very occasional cell labels *in vitro* with thymidine However 3 days after birth many large basophilic cells are found 20% of which are in DNA synthesis Similar mechanisms might underlie the findings of the present study preliminary results in infants with bacterial infections demonstrating increased numbers of cells in DNA synthesis and increased rates of lymphocyte RNA synthesis (2) indicate that the circulating lymphocytes of the newborn do react to microbial stimulation.

The magnitude of the response can not be quantitated by the methods employed in the present study since larger lymphocytes have a short transit time in the blood this is in contrast to small lymphocytes that include cells with immunological memory which remain in circulation between blood and lymphatic tissues for extended periods (6) It is noteworthy that the concentration of small lymphocytes in the blood increased steadily during the neonatal period (Fig. 1 B)

In order to clarify the development of lymphocyte responses during the neonatal period sequential in vitro-studies of their reactivity toward well-defined naturally occurring antigens will be of considerable interest.

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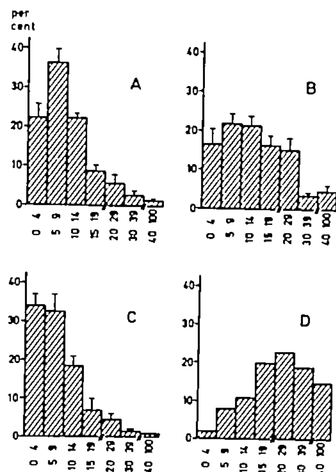


Fig 3 Uptake of H-cytidine by lymphocytes of infants Distribution of grain counts over 100 lymphocytes *Abscissa* Grain count classes (number of grains per lymphocyte) *Ordinate* Per cent lymphocytes in each grain count class (*A*) Average of 6 normal infants day 1 ±S E M (*B*) Average of 6 normal infants day 4 ±S.E.M (*C*) Average of 6 normal infants day 6 ±S E.M (*D*) Infant (5 days old) with bacterial infection (2)

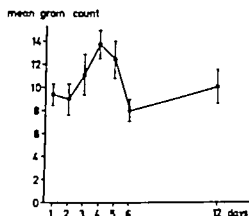


Fig 4 Average ^{3}H-cytidine labelling of lymphocytes of 6 normal infants Mean grain count per lymphocyte ±S E.M

lymphocytes were never found in DNA synthesis

In the ^{3}H-cytidine labelled preparations the number of cells with >100 grains agreed well with the number of labelled cells in the corresponding ^{3}H thymidine preparations and these cells have been omitted from the calculations The rate of incorporation of ^{3}H cytidine varied widely between individual lymphocytes (Fig 3) Small adult type lymphocytes showed slow cytidine uptake in the larger lymphocytes the grain count was not necessarily proportional to the basophilia of the cytoplasm

Cytidine incorporation activity increased from day 1 (Fig 3 A) to day 4 (Fig 3 B) and then again decreased (Fig 3 C) This is also illustrated if the course of the average mean grain count is plotted (Fig 4) In lymphocytes from infants with bacterial infections (2) increased cytidine uptake was found (Fig 3 D)

DISCUSSION

The number and possible functions of DNA synthesizing cells in human blood have been discussed by Killmann (10) In the normal human adult low numbers of thymidine-labelled lympho-plasmocytoid cells (0–8 per μl) and of monocytoid and blast like cells (0–4 per μl) are found The functions of these cells are not known with certainty However the available evidence is compatible with an immunological function of the lympho-plasmocytoid cells Their number increases in viral and bacterial infections in other conditions with acute inflammation (9) and following immunization with microbial antigens (4)

The studies by Winter et al (13) have shown that the number of lymphocytes of transitional type is high in the foetal circulation and that a sizeable proportion of these is in DNA synthesis at birth The functions of the DNA synthesizing cells are still de-

ated and the possibility that some are early myeloid cells is not excluded

The changes in DNA-synthesizing cells after birth have not been studied before We found a decrease during the first few days after delivery (Fig. 2) from day 3 to day 6 the concentration rose tenfold and then again decreased Cytidine incorporation although not a quantitative measure of RNA synthesis (8) does give an indication of RNA metabolic activity in the cell This parameter showed changes (Fig 4) parallel to those in the number of DNA-synthesizing cells and actually preceding these by one or two days an interval which is also encountered when lymphocytes are stimulated by antigen *in vivo* or *in vitro* (12)

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PLATELET FUNCTIONS IN CHILDREN WITH CONGENITAL HEART DISEASE

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ABSTRACT Goldschmidt, B. (Second Department of Paediatrics, Semmelweis Medical University Budapest, Hungary). Platelet functions in children with congenital heart disease. Acta Paediat Scand, 63: 271 1974.—Examinations of platelet functions (bleeding time, capillary resistance clot retraction, thrombocyte adhesiveness in vitro and in vivo, spontaneous platelet aggregation, ADP and collagen induced platelet aggregation) were performed in children with CHD In the cases with acyanotic heart disease (30 cases) the platelet functions were normal. The majority of cases with cyanotic CHD (35 cases) are thrombocytopenic. In these cases protracted bleeding time, increased capillary fragility and reduced clot retraction may be observed. The pathologic symptoms are connected with the low number of platelets. In patients with cyanotic CHD the platelet adhesiveness shows in vitro and in vivo a statistically significant increase In one third of cases, platelet aggregation is also increased in cyanotic patients. Following the administration of ADP and collagen the aggregation shows a normal course.

KEY WORDS: Congenital heart disease, platelet functions

In some of the children affected with congenital cardiac defects clotting disturbances may be observed (10 12) The hemostatic balance is shifted now in thrombotic then in hemorrhagic direction. The plasma level of coagulation factors is lower (8 19) the fibrinolytic activity is increased (9) the level of fibrinogen degradation products is raised (21) and the number of the platelets is frequently reduced (1 10 15 16 19 22 26) According to the clinical picture and laboratory findings clotting disturbance is a chronic disseminated intravascular coagulation (DIC) (10 19) The maintenance of hemostasis depends on the normal function of thrombocytes. Numerous disturbances of platelet function may be involved in the development both of increased coagulability and hemorrhagic diathesis. Our objective was to elucidate the question whether deviation in platelet function plays a role in the pathogenesis of clotting disturbance in children with congenital heart disease (CHD)

MATERIALS AND METHODS

Examinations were performed on 65 children suffering from various forms of cardiac malformation. Children of both sexes were represented aged between 3 and 14 years. Acyanotic CHD was present in 30 children (left-right shunt), while 35 were cyanotic (right–left shunt). The packed cell volume of acyanotic children was between 38 and 45% while in the cyanotic group it was between 48 and 90%. The oxygen saturation of arterial blood in acyanotic children exceeded 95% while in the cyanotic group the value was between 40 and 90% During and several weeks prior to the examinations, the children were not given drugs influencing coagulation or platelet functions.

Examinations were carried out in the late morning hours. Sampling of blood was performed from the cubital vein without stasis, with siliconed needles, and collected in polyester plastic tubes. Whole blood was anticoagulated with 1/9 volume of 3 8% disodium citrate. The citrate in cases of polycythemia was corrected ac

cording to the packed cell volume in order to stabilize the ratio of plasma citrate (8)

Platelet rich plasma (PRP) was obtained by centrifugation of citrated blood for 5 min at 800 rpm *platelet poor plasma* (PPP) by centrifugation of PRP for 15 min at 3500 rpm The platelet count in PRP was adjusted with PPP to 400000/mm³

Hematocrit was determined in microhematocrit tubes

Platelet counts were performed in double blind by phase contrast microscopy by procedure of Feissly & Lüdin (7)

Bleeding time was determined by the Ivy technique (20)

Capillary fragility and resistance was measured by the method of Rumpel Leede (?).

Clot retraction was determined in whole blood by the method of Macfarlane and in PRP by the method of Bettex-Galland & Lüscher (3) The clot retraction was measured as extracorpuscular clot volume (ECV)

In vivo platelet adhesiveness (platelet consumption on the vascular wound) was measured by the method of Borchgrevink and the results are expressed in percentage of platelets consumed (4)

In vitro platelet adhesiveness to glass was measured by the method of Hellem (18) After standing at room temperature for 30–45 min the citrated blood was passed through a standardized glass bead column The contact time between glass and blood was 30 sec Platelet adhesion was expressed according to the formula

$$\frac{\text{Initial count} - \text{final count}}{\text{Initial count}} \times 100\,\%$$

Platelet aggregability was determined in citrated PRP at 37°C using the method of Breddin (6). Depending on the number of aggregates we distinguish 5 stages Stages 1 and 2 are considered as normal while stages 3 to 5 are pathologic showing increasing aggregation of platelets

ADP induced and *collagen-induced platelet aggregation* was measured according to Breddin (6) Platelet aggregation was tested by adding ADP (final concentration 10 μg/ml) or connective tissue suspension (final concentration 6 0 mg/100 ml protein) to PRP and the mixture was agitated at 37°C

Adenosin diphosphate (ADP Chinoin Budapest, Hungary) a stock solution of 4 7×10⁻⁴ M was prepared in 0 15 M NaCl and stored at −20°C

Connective tissue suspension was prepared from subcutaneous fat according to Zucker & Borrelli (27) and stored at −20°C It was diluted 1 3 in buffer before use

With the exception of glass beads for platelet adhesiveness silicone glass instruments were used for investigations (Silicone Lubricant Dow Corning Corp Midland Mi USA)

The evaluation of the results was carried out by means of the Student s *t*-test χ^2-test and the correlation coefficient A *p* value of less than 0.05 was considered significant

RESULTS

The *platelet count* was within the normal limits (125000–400000/mm³) in acyanotic children suffering from cardiac disease The average value was 237000/mm³ (Fig 1) Thrombocytopenia was observed in the cyanotic patients In this group the average platelet count was 100000/mm³ (Table I)

Bleeding time was within normal limits of 2–8 min excepting 3 cases in acyanotic patients (Fig 1) The average in this group was 6 min In more than a half of cyanotic patients (23/35) we observed a protracted bleeding time The average values was 9 min (Table 1) Protracted bleeding time was due to thrombocytopenia (Fig 2A)

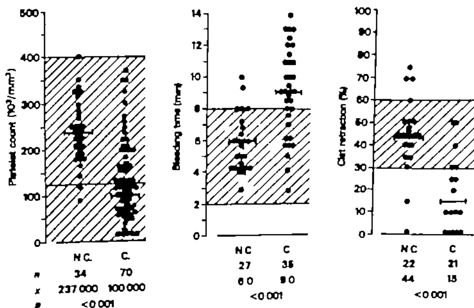


Fig 1 The platelet count, Ivy bleeding time and whole blood clot retraction in acyanotic (N C) and in cyanotic (C) congenital heart disease N = number of patients x̄ = mean values, *p* = level of significance Shaded areas indicate the normal range

Table 1 *Platelet functions in patients with congenital heart disease*

n=number of cases, x̄=mean value, S D =standard deviation

	Congenital heart disease						
	Acyanotic			Cyanotic			Significance
Test	*n*	*x*	S D		*x*	S D	*p*
Platelet count, 10^3/mm^3	34	237	±56	67	100	±48	<0.001
Bleeding time min	27	6.0	±2.2	35	9.0	±2.8	<0.001
Retraction of whole blood clot, %	22	44.5	±17	21	15.5	±17	<0.001
Adhesiveness in vivo %	24	38.0	±10.9	31	47.0	±12.6	<0.05
Adhesiveness in vitro %	24	36.0	±12.6	31	60.0	±15.6	<0.001

The *Rumpel-Leede's test* in 23 of the 29 acyanotic patients was normal (Table 2). In most of the cyanotic patients (27/36) increased capillary fragility was demonstrable. The increased capillary fragility is a consequence of the low number of platelets. Excepting four cases it was always in thrombocytopenic patients that the tourniquet-test proved to be positive (Fig. 2B).

Whole blood retraction was normal (between 30 and 60%) in acyanotic patients (Fig. 1). In cyanotic patients retraction of coagulum was frequently not observable. Concerning thrombosthenin function however this difference cannot be evaluated since owing to the high hematocrit and thrombocytopenia the absence of coagulum contraction may be secondary. Retraction was therefore also examined in the PRP. With the exception of few cases normal values were obtained in both groups.

Platelet adhesiveness in vivo was normal in acyanotic patients (Fig. 3B). During wounding 20 to 50% of platelets adhered to the released interstitial fibres. The average value was 38%. As for the cyanotic patients in 11 of 31 cases increased adhesivity was observed.

Platelet adhesiveness in vitro in acyanotic patients excepting 5 cases was within normal

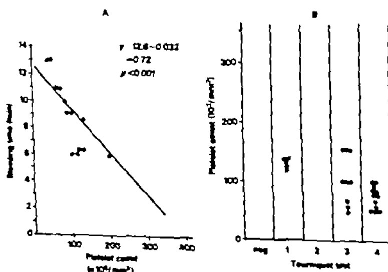


Fig. 2. Relationship between platelet count and Ivy bleeding time (A) and relationship between platelet count and capillary fragility (B) in cyanotic congenital heart disease.

Table 2 *Rumpel Leede test in children with congenital heart disease*

Group	No of cases	Capillary resistance test stages 0	+	2+	3+	4+	χ^2	p
Acyanotic	29	13	10	6	–	–	19 67	<0.001
Cyanotic	36	–	9	3	11	13		

limits (20 to 50%) (Fig 3 A) On average 35% of platelets adhered to the glass surface In the cyanotic group an increased adhesivity was found in most of the cases (20/31) The average value was 60% (Table 1)

Thrombocyte aggregation was normal in 20 of 26 acyanotic and in 20 of 35 cyanotic children (Table 3) In the others the aggregation of platelets was increased Platelet aggregation in presence of ADP resp collagen was normal in every case

DISCUSSION

In children with congenital heart disease without cyanosis a coagulation anomaly is relatively infrequent (10 22) In these patients platelet functions were also normal

In children with cyanotic CHD and chronic hypoxemia clotting disturbances are frequent (1 10 17 19 22) This according to our observations applies also to the platelet functions The deviations may be classified in two groups

Ad 1 Disturbances such as protracted bleeding time increased fragility of vessel wall imperfect or absent contraction of whole blood clot developing as a consequence of thrombocytopenia These phenomena are secondary and rather of the quantitative then qualitative changes of platelets

Ad 2 Thrombocyte function disturbances which are the consequence of pathologic cellular function increased aggregability of platelets and increased adhesiveness of platelets in vivo and in vitro to foreign surfaces These deviations are independent of the number of circulating platelets

The low platelet count in patients with cyanotic CHD was noted 20 years ago (1 17) Since that time many authors were interested in this phenomen (10 15 19 22 26) the pathogenesis however remained unknown Theoretically there may be two ways for the development of thrombocytopenia it might be due to reduced production or increased disappearance In hypoxemic cyanotic cardiopathic children we observed earlier that the medullary megakaryocyte/thrombocytopoiesis is normal and regulated according to the peripheral requirements by a plasma factor the thrombopoietin (11) Kummer et al (23) showed that the life span of platelets is shorter than normal in cyanotic

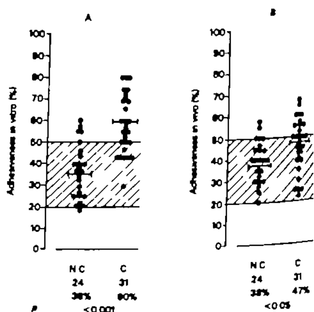


Fig 3 Platelet adhesiveness in vitro (A) and in vivo (B) in acyanotic (N C.) and in cyanotic (C) congenital heart disease *n* = number of patients *x* = mean values, *p* = level of significance Shaded areas indicate the normal range

Table 3 *Platelet aggregation tests (spontaneous ADP and collagen-induced aggregation) in children with acyanotic and cyanotic heart disease*

Test	Group of patients	No of cases	Platelet aggregation stages					χ^2	p
			1	2	3	4	5		
Platelet aggregation	acyanotic	26	11	9	5	1	–	4.25	<0.05
	cyanotic	35	10	10	6	6	3		
ADP-induced aggregation	acyanotic	26		–	–	10	16	1.06	<0.05
	cyanotic	35	–	–	–	17	16		
Collagen induced aggregation	acyanotic	26	–	–	6	8	12	0.049	<0.95
	cyanotic	33	–	–	–	15	18		

patients. Owing to this observation we assume that an increased peripheral consumption might induce thrombocytopenia. The present investigations support our earlier conceptions. The increased aggregation and adhesiveness of platelets from the pathologic basis for the conglutination and adhesion on microcirculatory areas and for the disappearance from circulation.

Susceptibility to thrombosis and hemorrhagic diathesis represent the two extremes of the disintegrated *hemostatic balance*. In cyanotic patients hence DIC and consumption coagulopathy representing a pathologic unit today may already be the same denomination. Earlier only the laboratory signs of the second and third step of the phenomenon —chronic consumption and hyperfibrinolysis —could be observed.

It is our opinion that, in patients with cyanotic CHD the increased susceptibility to aggregation and adhesion of platelets is the first step which through the adhesion of platelets to the vessel wall leads to the development of DIC. Thrombus *formation* of course should be considered as a pluricausal pathologic phenomenon. The behaviour of platelets is only one though rather important factor in the pathogenesis. The other factor is the slowing down of blood flow caused by increased blood *viscosity (due to polycythemia)* and the *mechanic-hemodynamic insufficiency of cardiac action*. Due to the slower circulation and to hypoxemia damages of the vessel wall occur (16). Intravasal thrombus formation actually starts after the release reaction of platelets adhering to the injured vascular endothelium and to the released subendothelial connective tissue. It may be assumed than in the majority of cases the increased fibrinolytic activity—developing compensatorily as a result of damaged vessel wall and thrombus formation—inhibits extension of the process and restores the disintegrated hemostatic balance (9). Manifest thrombus resp hemorrhage develops only in imperfectly or overcompensated cases.

The aggregation and adhesiveness of platelets is influenced by several factors. In cyanotic patients a close connection was observed in vivo and in vitro between the increased stickiness of platelets hypoxemia, polycythemia and hyperlactacidemia (13, 14).

On the basis of our present studies it might be possible that the disturbed hemostasis of cyanotic cardiac patients may be prevented by the drugs decreasing adhesivity and aggregation of thrombocytes.

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NECROTIZING ENTEROCOLITIS AFTER CATHETERIZATION OF THE UMBILICAL VESSELS

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ABSTRACT Livaditis, A., Wallgren, G and Faxelius, G. (Departments of Pediatrics and Pediatric Surgery Karolinska Sjukhuset, Stockholm, Sweden). Necrotizing enterocolitis after catheterization of the umbilical vessels. Acta Paediat Scand, 63: 277 1974.— Necrotizing enterocolitis is reported in 7 infants who had undergone umbilical vessel catheterization. Five of them were treated surgically with two fatalities, while the remaining two recovered following conservative management. Necrotizing enterocolitis is potential complication of umbilical vessel catheterization in particularly predisposed infants. The disorder may occur regardless of the type of vessel used or the indications for the procedure Unless the ductus venosus is easily passed, umbilical venous catheterization for cardiovascular studies should preferably be avoided

KEY WORDS: Newborn, umbilical vessel catheterization, exchange transfusion, necrotizing enterocolitis

In the neonatal period the umbilical vessels are commonly used for exchange transfusion fluid administration and blood sampling for the assessment of cardiopulmonary disease. Since this vascular route is amenable to cardiac catheterization it has also been suggested for the study of suspected cardiac anomalies (7 14 17 24) In recent years reports of complications presumably related to catheterization of the umbilical vessels have appeared in the literature with increasing frequency (1 3 6, 11 16, 20 25 28 29) A well-recognized complication following exchange transfusion through the umbilical vessels is necrotizing enterocolitis which may lead to bowel perforation and fatal peritonitis. Although the Pediatric Clinic at Karolinska sjukhuset due to centralized care of erythroblastotic infants, has had annual average totals in excess of 300 exchange transfusions complications from the gastrointestinal tract have not been observed earlier In the last few years however necrotizing enterocolitis was encountered in 7 infants who had undergone umbilical vessel catheterization for various reasons in this hospital Five of these required operation while two made a slow recovery without surgical intervention The seemingly high incidence of this complication has prompted the present report to be added to the numerous similar presentations in the literature

CASE SUMMARIES

The 7 cases of necrotizing enterocolitis are summarized in Table 1 There were 4 males and 3 females four of them were prematures. The indications for umbilical vessel catheterization were exchange transfusion in 4 cases and acute respiratory distress due to either congenital heart disease or neonatal asphyxia in 3 cases. In 4 infants the umbilical vein alone was used, in two both the vein and artery were entered and in one the artery alone was employed Of the 4 infants who were treated

Table 1 *Clinical data*

Case no	Sex	Body weight (g)	Indic for umb cath	Vessel used	Age at onset of symptoms (days)	Time after cath (days)	Clinical features	X-ray findings
1	m	2 850	E T ×6	UV	7	1–6	Acutely ill vomiting abd distension	Pneumoperitoneum & peritoneal fluid
2	f	2 470	E T ×1	UA	6	2	Acutely ill vomiting abd distension edema of lower extr blood p r	Pneumoperitoneum & peritoneal fluid
3	f	2 180	E.T ×4	UV	3	1–2	Acutely ill vomiting abd distension	Dilated loops of small bowel
4	m	4 100	IRDS+ CHD	UV+UA	6	2	Acutely ill vomiting abd distension blood p.r	Pneumatosis intest.
5	f	2.160	IRDS	UV+UA	2	2	Acutely ill vomiting abd distension	Pneumoperitoneum & peritoneal fluid
6	m	3 900	IRDS+ CHD	UV	14	12	Vomiting abd distension blood p r	Pneumatosis intest
7	m	2 190	E.T ×1	UV	7	1	Acutely ill vomiting abd distension blood p r	Pneumatosis intest

with exchange transfusion one had the procedure performed six times one four times and two once each

The age at onset of symptoms of necrotizing enterocolitis ranged from two days to 2 weeks All of the infants were severely ill Vomiting and abdominal distension were the most prominent findings and were invariably present. Four infants passed blood per rectum and in one of them pieces of necrotic mucosa were found in the bowel discharge Edema of the flanks and lower extremities was observed in one of the patients Upon roentgenographic examination pneumatosis intestinalis was evident in 3 cases and pneumoperitoneum with intraperitoneal fluid was found in 3 cases In one additional case flat film of the abdomen showed dilated loops of small bowel but no free gas This finding was interpreted as intestinal obstruction due to meconium plug syndrome and prompted gastrografin enemas which however proved ineffective

Five of the 7 infants were operated upon (Table 2) The ages of the patients at the time of operation ranged from 3 to 8 days Colonic perforation with resultant widespread peritonitis was found in 3 patients but the remaining 2 had no gross evidence of perforation In one of them the necrotizing process involved the entire colon and in the other the entire small bowel In the latter case the necrotic bowel was in several areas paper-thin and disintegrated whenever attempts to mobilize it were made Subtotal colectomy closure of the rectal stump and ileostomy was performed in 3 patients. All of them survived and were successfully reoperated 6 months later with an ileorectal anastomosis One patient with cecal perforation was operated with a cecostomy The postoperative course was complicated by anuria increasing jaundice generalized edema, bleeding tendency and cardiac arrest which led to death on the 4th postoperative day Autopsy revealed widespread peritonitis and circumscript areas of small bowel wall necrosis

In our 3rd case the severity and extent of the lesion precluded any form of reconstructive surgery The abdominal wound was closed In view of the hopeless prognosis no attempts at supportive fluid therapy were made postoperatively Death occurred on the 13th postoperative day because of septic peritonitis and severe cachexia. Autopsy revealed gangrene with multiple perforations and fistula formation involving approximately 2/3 of the small bowel The remaining small bowel and colon appeared to be grossly normal

Two infants were treated conservatively with nasogastric suction intravenous fluids and antibiotics. Following this regimen they made a satisfactory recovery and were discharged at the age of 4 weeks Follow-up barium enema studies were unremarkable

DISCUSSION

Necrotizing enterocolitis is a well recognized entity occurring usually during the first few

ble 2. *Operative treatment and results*

Operative findings	Procedure	Results	Autopsy findings	Comment
Perforation of cecum	Cecostomy	Died	Diffuse peritonitis, distinct areas of small bowel wall necrosis	
Perforation at splenic flexure necrotizing inflammation of entire colon	Subtotal colectomy, ileostomy and closure of rectal stump	Recovered		Ileorectal anastomosis at the age of 6 months. Alive and well
Necrotizing process involving the entire small bowel	Exploratory laparotomy	Died	Gangrene of about 2/3 of small bowel. Remaining bowel and colon of normal appearance	Survived 13 days following operation without oral or parenteral nutrition
Necrotizing inflammation of entire colon	Subtotal colectomy, ileostomy and closure of rectal stump	Recovered		Ileo-rectal anastomosis at the age of 6 months. Alive and well
Multiple colonic perforations	Subtotal colectomy, ileostomy and closure of rectal stump	Recovered		Ileorectal anastomosis at the age of 6 months. Alive and well

days of life predominantly in prematurely born infants. Although this lesion may involve any part of the gastrointestinal canal it shows a definite predilection for the colon. The affected bowel wall exhibits areas of necrosis, ulceration and inflammatory reaction (23) the gross and microscopic tissue appearance being strikingly similar to that of ischemia. In spite of numerous attempts to explain the cause of necrotizing enterocolitis its background has not been definitely proved. Pathogenetic suggestions have centered mainly along three lines, namely vascular occlusive disease, immunopathologic reaction and infection by specific micro-organism (8, 12, 13, 19, 22, 30, 31). Since the first description of this disorder in a newborn (10) prematurity has been considered an important predisposing factor.

Recent reports on necrotizing enterocolitis in association with exchange transfusion have suggested a more than coincidental connection with this procedure (? 4, 9, 15, 18, 21). Investigations of the hazards of umbilical vessel catheterization *per se* have demonstrated an amazingly high incidence of local complications such as thrombus formation, particularly in conjunction with umbilical venous catheterization (28). These observations added to the fact that the catheter tip during exchange transfusion is usually lodged near the portal sinus, seem to favour the opinion that the gastrointestinal complication may be of circulatory or thrombo-embolic origin. A thromboembolic process may be induced retrogradely over the portal veins or antegradely over the ductus venosus and foramen ovale to the systemic circulation.

All of our patients were catheterized by the umbilical route. In 4 of them only the umbilical vein was used. In 2 both the artery and vein were entered and in one, in whom the venous approach had failed, the artery alone was employed. Of the 4 infants who were treated with exchange transfusion, one had the procedure performed six times and another four times. Both of these infants exhibited attacks of cardiorespiratory distress during one of the exchange transfusions necessitating temporary arrest of the procedure.

for resuscitation Diagnostic catheterization in 2 additional patients presented difficulties when efforts were made to pass the ductus venosus to the heart and the portal veins were repeatedly entered before the catheter was eventually slipped through the ductus The only infant who had umbilical catheterization for fluid-buffer administration was in acute distress prior to this procedure Radiological examination in this case showed signs of necrotizing enterocolitis immediately after birth suggesting that the process had started antenatally

Although it is difficult retrospectively to appreciate fully the clinical data in detail it seems clear that all of these patients prior to or in connection with umbilical catheterization were severely ill In 5 of them the procedure was either complicated or judged to be difficult The reportedly low incidence of serious complications of umbilical catheterization suggests that the procedure itself only serves as a trigger in particularly predisposed infants whose underlying condition provides presumably the necessary prerequisite factor(s) This idea is further supported by the fact that many cases of necrotizing enterocolitis unassociated with umbilical catheterization have been described in infants suffering from various neonatal conditions (19)

Necrotizing enterocolitis is a neonatal abdominal emergency carrying a grave prognosis Therefore immediate diagnosis and prompt treatment are imperative for a favorable outcome The disorder should be suspected in any infant who following umbilical vessel catheterization develops vomiting progressive respiratory distress and abdominal distension When in addition signs of sepsis and shock with passage of bloody stools become evident this condition should be seriously considered X ray films of the abdomen invariably provide reliable diagnostic information and indeed they may be positive before the clinical syndrome is fully developed The earliest characteristic finding is pneumatosis intestinalis occuring in about 97% of the cases (27) Occasionally intestinal distension with fluid levels may be found prior to pneumatosis intestinalis but this is not a specific feature of the disease Evidence of pneumoperitoneum suggesting perforation of gangrenous bowel is usually a late finding. Barium enema studies should be omitted because they do not add to the diagnosis and entail the risk of perforation Abdominal paracentesis which has been advocated by some authors (30) does not seem to have a place in the diagnosis Therapeutic aspiration of free intra abdominal gas however may be indicated in cases with severe respiratory distress

Unless frank perforation is present, initial treatment of necrotizing enterocolitis should preferably be conservative Therapy should include intestinal decompression parenteral fluids and antibiotics Occasionally blood oxygen vitamin K and eventually corticosteroids may be required Frequent clinical and radiological assessment of the patient s condition is crucial during this period of watchful waiting A surgeon should be consulted immediately and with the pediatrician should observe the patient carefully for any evidence of progression of the process Approximately 40% of the infants may respond satisfactorily to this regimen and recover without emergency operation (27) Our experience though limited seems to support this statement as conservative management proved successful in 2 of our 7 cases Since neither of these infants was subjected to laparotomy it can be argued that the diagnosis was not confirmed However these patients histories clinical signs and radiological findings so closely resembled those of the other cases in this series that no doubt remains as to the accuracy of the diagnosis

Ideally patients with necrotizing enterocolitis should be operated upon before bowel perforation takes place The indications for surgery prior to perforation may be difficult to establish inasmuch as there is not always

definite correlation between clinical and pathologic findings (5) As a rule increasing deterioration of the infant's general condition suggesting sepsis or peritonitis should be an indication for emergency laparotomy We feel that it is better to perform occasionally an unnecessary laparotomy than to wait until perforation and fatal peritonitis develop

The type of operation depends upon the location extent and severity of the lesion Subtotal colectomy with closure of the rectal stump and ileostomy was successfully used in 3 of our patients exhibiting extensive colonic involvement. In all of them a secondary ileorectal anastomosis could be performed without difficulty In our opinion this two-stage approach is safer than a direct anastomosis of questionable viability which in addition must be done in a highly contaminated field Careful inspection of the entire gastrointestinal canal should be made at operation to ascertain that the disorder does not involve several areas In one of our fatal cases the disease was thought to be confined to the cecum which was exteriorized At autopsy however multiple areas of necrosis were found in the small bowel Total involvement of the intestinal canal is a hopeless condition usually providing no means for surgical treatment. Nevertheless a second look should be made a few days after laparotomy to establish whether demarcation of the process occurred which might permit surgical relief This concept is supported by the autopsy findings in one of our patients which showed that segmental regression of the initially extensive lesion had actually occurred.

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for resuscitation Diagnostic catheterization in 2 additional patients presented difficulties when efforts were made to pass the ductus venosus to the heart and the portal veins were repeatedly entered before the catheter was eventually slipped through the ductus The only infant who had umbilical catheterization for fluid-buffer administration was in acute distress prior to this procedure Radiological examination in this case showed signs of necrotizing enterocolitis immediately after birth suggesting that the process had started antenatally

Although it is difficult retrospectively to appreciate fully the clinical data in detail it seems clear that all of these patients prior to or in connection with umbilical catheterization were severely ill In 5 of them the procedure was either complicated or judged to be difficult The reportedly low incidence of serious complications of umbilical catheterization suggests that the procedure itself only serves as a trigger in particularly predisposed infants whose underlying condition provides presumably the necessary prerequisite factor(s) This idea is further supported by the fact that many cases of necrotizing enterocolitis unassociated with umbilical catheterization have been described in infants suffering from various neonatal conditions (19)

Necrotizing enterocolitis is a neonatal abdominal emergency carrying a grave prognosis Therefore immediate diagnosis and prompt treatment are imperative for a favorable outcome The disorder should be suspected in any infant who following umbilical vessel catheterization develops vomiting progressive respiratory distress and abdominal distension When in addition signs of sepsis and shock with passage of bloody stools become evident this condition should be seriously considered X ray films of the abdomen invariably provide reliable diagnostic information and indeed they may be positive before the clinical syndrome is fully developed The earliest characteristic finding is pneumatosis intestinalis occuring in about 97% of the cases (27) Occasionally intestinal distension with fluid levels may be found prior to pneumatosis intestinalis but this is not a specific feature of the disease Evidence of pneumoperitoneum suggesting perforation of gangrenous bowel is usually a late finding. Barium enema studies should be omitted because they do not add to the diagnosis and entail the risk of perforation Abdominal paracentesis which has been advocated by some authors (30) does not seem to have a place in the diagnosis Therapeutic aspiration of free intra-abdominal gas however may be indicated in cases with severe respiratory distress

Unless frank perforation is present, initial treatment of necrotizing enterocolitis should preferably be conservative Therapy should include intestinal decompression parenteral fluids and antibiotics Occasionally blood oxygen vitamin K and eventually corticosteroids may be required Frequent clinical and radiological assessment of the patient's condition is crucial during this period of watchful waiting A surgeon should be consulted immediately and with the pediatrician should observe the patient carefully for any evidence of progression of the process. Approximately 40% of the infants may respond satisfactorily to this regimen and recover without emergency operation (27) Our experience though limited seems to support this statement as conservative management proved successful in 2 of our 7 cases Since neither of these infants was subjected to laparotomy it can be argued that the diagnosis was not confirmed However these patients histories clinical signs and radiological findings so closely resembled those of the other cases in this series that no doubt remains as to the accuracy of the diagnosis

Ideally patients with necrotizing enterocolitis should be operated upon before bowel perforation takes place The indications for surgery prior to perforation may be difficult to establish inasmuch as there is not always

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CASE REPORT

CARDIAC RHABDOMYOMA IN INFANCY

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ABSTRACT. Harinck, E., Moulaert, A. J. M. G., Rohmer, J. and Brom, A. G. (Departments of Paediatric Cardiology and Thoracic Surgery, University Hospital, Leiden, The Netherlands). Cardiac rhabdomyoma in infancy. Acta Paediat Scand, 63: 283, 1974.—The clinical picture of three cases of cardiac rhabdomyoma is presented. Surgical removal was attempted in two cases. One of them is still alive 9 years after the operation but has a severe mental retardation due to the associated tuberous sclerosis. Particular attention is given to the frequent association of cardiac rhabdomyoma and tuberous sclerosis. With the progress of cardiac surgery rhabdomyomas of the heart often can be removed successfully but surgical intervention is justified only in the absence of tuberous sclerosis or brain damage.

KEY WORDS: Cardiac rhabdomyoma, tuberous sclerosis

Although cardiac rhabdomyomas are probably the commonest cardiac tumour in infancy (16) the majority of cases reported are diagnosed at autopsy. The number of cases diagnosed alive is very limited (6, 8, 13, 16, 17, 18, 19). So far as we know only two successful operations of this type of tumour have been described (6, 16, 17). The reason for adding three cases of cardiac rhabdomyoma to the literature list of approximately one hundred cases is primarily to present a 9-year-old-boy who underwent a surgical resection of two large left ventricular rhabdomyomas at the age of 4 months. Furthermore, this article may serve to emphasize that cardiac rhabdomyomas may be diagnosed during life with reasonable accuracy.

Case Report

Case 1

A 4-month-old-boy was admitted to the Leiden University Hospital in 1963 for convulsions, feeding difficulties and failure to thrive. He was born by caesarian section after a normal pregnancy. Pallor and asphyxia were present immediately after birth. The baby showed frequent generalized convulsions during the first 3 days of life but they disappeared spontaneously. Convulsions recurred at the age of 2 months. Clinical examination revealed a normal looking infant. Weight and length were according to age. All pulses were palpable and normal. Cardiac dullness extended almost to the left anterior axillary line. Auscultation revealed an accentuated single second sound but no murmurs. No neurological abnormalities were noticed. The electrocardiogram was compatible with left ventricular hypertrophy and ischaemia. Roentgenograms revealed extreme enlargement of the left ventricle. Cardiac catheterization was not done. The diagnosis of an abnormal origin of the left coronary artery was suspected. Thoracotomy was performed and it revealed a normal origin of the coronary arteries. Two separate tumours were situated in the apex of the left ventricle. One of these tumours extended as far as the right ventricular endocardium (Fig. 1).

Both tumours were removed. The microscopic findings of these masses established the diagnosis cardiac rhabdomyoma. The postoperative period was uneventful. Pneumoencephalography was done and revealed cerebral intraventricular masses which were very suspect of tuberous sclerosis. As in most cases of tuberous sclerosis, gradual but severe mental retardation resulted. Eight years after the operation, cardiac catheterization showed

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No neurological abnormalities were found. The chest X ray films showed massive cardiomegaly and pulmonary plethora. The electrocardiogram showed intraventricular conduction disturbances. Cardiac catheterization revealed a large left-to-right shunt through an atrial septal defect. Angiocardiography confirmed this shunt. In the left ventricle, near the mitral orifice a space-occupying lesion was present which unfortunately was not seen prior to death. The patient died of severe heart failure. Autopsy revealed a large mass in the left atrial and left ventricular wall, almost occluding the mitral orifice. There was also a large defect in the inferior and dorsal atrial septum. Microscopic examination established the diagnosis cardiac rhabdomyoma

DISCUSSION

Cardiac rhabdomyomas have a strong predilic tion for the very young (12, 15) Therefore it is not surprising that our 3 cases concerned infants Manifestations of tuberous sclerosis (1 3 11 12) commonly associated with this type of tumour were observed in the first case Signs of tuberous sclerosis are not commonly present in the very young, therefore it is *understandable that they were absent in* cases 2 and 3

Autopsies of the brains were not performed Repolarization disturbances were seen on all three electrocardiograms but cardiac dysrhythmias commonly seen in these cases (2, 4 5 9 13 15) did not occur

Anomalies such as renal tumours (12) adenoma sebaceum of the skin (15) congenital pulmonary involvement (7) also associated with cardiac rhabdomyomas were not found by autopsy in cases 2 and 3 In case 1 there still is no evidence of these anomalies.

Although these tumours are often located in the left ventricle (15) the clinical picture of a haemodynamically significant subaortic stenosis in early infancy due to a cardiac rhabdomyoma (case 3) appears to be rare No more than three cases have been reported (10 16 17). Despite the fact that a rhabdomyoma is probably the most common type of cardiac tumour in infancy (16) the clinical diagnosis is often missed because one does not think of it.

As most patients with cardiac rhabdomyoma die during infancy the authors feel that extensive diagnostic evaluation, *including cardiac* catheterization and angiocardiography early in life is indicated In view of the few successful operations of cardiac rhabdomyomas carried out in infancy and considering the very limited experience of the authors stringent indications for operation cannot be given. Shaher et al (16 17) suggest that resection of the tumour should be carried out if possible, when it seriously impedes cardiac function The good results with open heart resection of other primary cardiac tumours (14) may substantiate Shaher s suggestion. This means that some infants who normally would die may remain alive with a severe mental retardation as a result of the associated tuberous sclerosis There fore we feel that resection of the tumour should be attempted only when tuberous sclerosis is not suspected and when there is no evidence of brain damage

If these indications for surgery had been applied in our first case surgical treatment might *not have been undertaken Unfortunately* tuberous sclerosis was not suspected before the operation A more definite opinion regarding the surgical indications may be given when more results are reported

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Fig 1 Case 1 Two cardiac rhabdomyomas *in situ* at operation. The drawing shows the location of the left ventricular masses.

normal pressures in the left atrium, left ventricle and aorta. Left ventricular angiocardiograms revealed a slight enlargement of the left ventricle with a small aneurysm in the inferior and lateral aspect. No space occupying lesions were seen.

Case 2

A 2-day-old-boy was admitted to the University Hospital because of cyanosis and progressive dyspnoea. Pregnancy and delivery were uneventful. Clinical examination revealed a cyanotic infant with severe dyspnoea. The heart sounds were muffled and a grade 2/6 pansystolic blowing murmur was heard. The liver was slightly enlarged. The peripheral pulses were hardly palpable. There were no abnormal neurological findings. Routine laboratory investigations revealed a metabolic acidosis. Chest X-ray showed severe cardiomegaly with a normal pulmonary vasculature. The electrocardiogram was compatible with left ventricular hypertrophy. At cardiac catheterization extremely high pressures in the left atrium and the left ventricle were found. The descending aorta was reached through a patent ductus arteriosus. Cinéangiography from the pulmonary artery showed a right-to-left shunt through the patent ductus arteriosus. Injection into the left ventricle revealed a mitral insufficiency and a severe subaortic stenosis. Emergency thoracotomy was performed. An arteriotomy in the aorta showed a normal aortic valve. Below the valve a greyish homogenous mass was present and removed as completely as possible. Shortly before closure of the chest irreversible ventricular fibrillation occurred and the child died.

Autopsy revealed the remnants of a mass, below the aortic valve, extending into the anterior leaflet of the mitral valve. The mitral valve was not obstructed. There was a patent foramen ovale and a patent ductus arteriosus. Microscopic examination of the tumour established the diagnosis cardiac rhabdomyoma.

Case 3

A 14-day-old-boy was admitted to the University Hospital for failure to thrive, pallor, feeding difficulties, dyspnoea and excessive sweating. Pregnancy and delivery were uneventful. The heart was enlarged. There was a grade 2/6 ejection murmur in the third intercostal space and a gallop rhythm. The liver and spleen were enlarged.

o neurological abnormalities were found. The chest X ray films showed massive cardiomegaly and pulmonary plethora. The electrocardiogram showed intraventricular conduction disturbances. Cardiac catheterization revealed a large left-to-right shunt through an atrial septal defect. Cineangiocardiography confirmed this shunt. In the left ventricle, near the mitral orifice, a space-occupying lesion was present which unfortunately was not seen prior to death. The patient died of severe heart failure. Autopsy revealed a large mass in the left atrial and left ventricular wall, almost occluding the mitral orifice. There was also a large defect in the inferior and dorsal atrial septum. Microscopic examination established the diagnosis cardiac rhabdomyoma

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CASE REPORT

A CASE OF FOCAL DERMAL HYPOPLASIA SYNDROME (GOLTZ) WITH BILATERAL CHEILO-GNATHO-PALATOSCHISIS

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ABSTRACT Valerius, N. H. (Department of Paediatrics G and Department of Paediatrics TG with Queen Louise's Children's Hospital, Rigshospitalet, Copenhagen, Denmark). A case of focal dermal hypoplasia syndrome (Goltz) with bilateral cheilo-gnatho-palatoschisis. Acta Paediat Scand, 63: 287 1974.—A case of focal dermal hypoplasia syndrome is reported. The patient presented with a bilateral cheilo-gnatho-palatoschisis, which has not previously been described in association with this syndrome A short description of the syndrome is given. It is likely that cheilo-gnatho-palatoschisis is an infrequent finding in focal dermal hypoplasia syndrome but at present there is no evidence to confirm or disprove this.

KEY WORDS. Focal dermal hypoplasia, Goltz's syndrome, cheilo-gnatho-palatoschisis

A syndrome with focal dermal hypoplasia and associated widespread congenital dysplasias of structures of ectodermal and mesodermal origin was described by Goltz et al. in 1962 (2) In 1970 nearly 50 cases had been reported (3)

In the following we shall describe a case in which a bilateral cheilo-gnatho-palatoschisis was observed a malformation not previously noted in association with this syndrome

CASE REPORT

A girl, born in August 1969 Both parents and an elder sister were in good health There were no genetic disorders or congenital malformations in the family The mother had had no abortions The mother's prepartum course was uneventful until 5 week prior to term at which time she was admitted because of a suspicion of placental dysfunction which was not confirmed The delivery at term was without complications, the neonate's weight being 1 850 g and her length 43 cm. She was awarded a 1 minute Apgar score of 10. The following malformations were noted.

(a) Diffusely scattered areas of epithelial defects throughout the body (Fig 1) (b) Bilateral cheilo-gnatho-palatoschisis (Fig 1) (c) Syndactylia of 4th and 5th phalanx of the right hand and foot. (d) Radial deviation of the carpo-phalangeal joint and hyperextension of the inter phalangeal joint of the left index finger (e) Absence of nails on the left thumb and index finger (f) Colobomas of the chorioidea of both eyes and coloboma of the iris on the right eye. Aniridia and ectopia of the lens of the left eye. (g) A convergent strabismus. (h) A small omphalocele (Fig. 1)

Biopsy of the skin from an area with epithelial atrophy showed atrophy of the dermis typical of focal dermal hypoplasia. Nothing abnormal was noted following X-ray of the chest, skeletal X-rays and intravenous pyelography Routine laboratory examinations including ECG and EEG were normal, as was a chromosome study

In August 1971 she was readmitted for an assessment. It was then noted that her physical and psycho-motor development were retarded. She weighed 7 200 g and was 75 cm tall. She was unable to sit alone and was able to stand only when supported. She could form no intelligible words but was able to smile The following additional defects were noted.

(i) Several herniations of subcutaneous fat through the dermis on her left leg (j) Several small papillomas on her left lower lip. (k) Her hair was unusually thin and sparse (l) The dentition was delayed and her teeth were

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CASE REPORT

KINKY HAIR SYNDROME

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ABSTRACT Møllekær A. M. (Department of Paediatrics and the Department of Neuropathology Århus University School of Medicine, Århus, Denmark). Kinky Hair Syndrome Acta Paediat Scand, 63:289 1974.—Two brothers with Kinky Hair Syndrome are described. Both of them had hypothermia. In the younger boy low levels of serum copper and ceruloplasmin were demonstrated. At autopsy the most surprising finding was a demyelinating process in the brain. The fact that the younger boy showed signs of Kinky Hair syndrome at birth makes it difficult to accept an intestinal malabsorption of copper as the only underlying defect in the disease.

KEY WORDS: Kinky Hair Syndrome, hair copper and ceruloplasmin, demyelination of the hemispheres

In 1962 Menkes et al. (10) described a new degenerative cerebral disease Five boys of the same family presented an identical clinical picture with early progressing psychomotor retardation failure to thrive convulsions and death in the 1st to 3rd year All of them had sparse coarse stubby and kinky hair The disease was supposed to be hereditary with a sex-linked recessive transmission Up to date 24 cases have been reported (1 2 3 4 5 8 10 14 15 17)

Characteristic skeletal changes and severe malformations of the cerebral arterial system and the extracerebral arteries have been described (5 16) Furthermore hypothermia, low levels of serum copper and ceruloplasmin and low copper content of liver tissue have been reported (4 5) Low copper levels have also been found in the urine and hair (15) On the other hand increased levels of copper in the gut mucosa have been demonstrated (6)

In the following the first two cases of Kinky Hair Syndrome (KHS) in Scandinavia will be published

Case Reports

The two brothers were the only children of healthy unrelated parents. The parents had normal hair and abnormalities in the hair shaft could not be demonstrated by ordinary light microscopical investigation. The serum copper and ceruloplasmin concentrations of both parents were normal

Case I

CÅT (210471/1467) (Fig 1) was the younger brother Pregnancy and delivery had been normal In particular no asphyxia was noticed. The birth weight was 2 840 g the length 48 cm. There was icterus from the 3rd to the 7th day with maximal serum bilirubin value of 18 mg/100 ml. When he was a few days old, a peculiar pale yellow skin, woolly hair in tufts and pectus carinatum were noticed.

At the age of 7 weeks he was admitted to the hospital because of failure to thrive and transitory jerks in the extremities. He was pale and thin, with sparse stubby kinky and fair hair and sparse eyebrows. The skin was dry but the nails normal. The skull was compressed sidewards and the palate high arched. The lower part of the sternum and the curvature were prominent The tonus

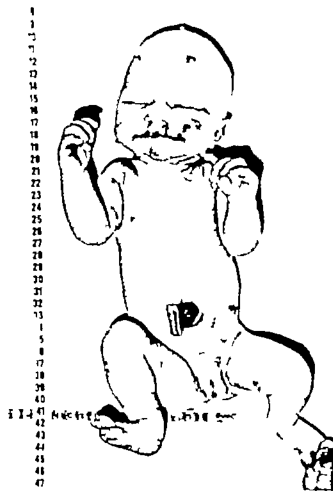


Fig 1 The patient s appearance at 2 days of age

hypoplastic with enamel defects (*m*) X-ray of the chest showed aplasia of the medial part of the right clavicle but was otherwise normal (*n*) Psychological assessment using the Cattell test revealed the patient to be quite retarded

Routine laboratory examinations including EEG and ECG were normal Skeletal X rays were normal

Her most recent evaluation occurred in May 1973 She weighed 8 300 g and had grown to a height of only 83 cm Her psycho-motor development continued to be very slow Her vision seemed to be rather good Apart from the development of a slight kypho-scoliosis there had been no progression of her symptoms

DISCUSSION

A number of surveys has been published on the focal dermal hypoplasia syndrome (2 3 4 6 7) Warburg (7) in recording the first Danish case also included descriptions of 32 cases previously published It appears from her review that 90% of the reported cases have been females In all patients malformations of the hands and feet are noted most often syndactylia and aplasia or hypoplasia of the phalanges and metacarpals Malformations of the eyes are very frequent especially colobomas or aniridia and microphthalmia. Also the teeth are frequently hypoplastic with defective enamel The typical skin-lesions consist of scattered areas of hypoplasia and aplasia of the skin hyperpigmentation and telangiectasias Microscopic examination of these areas shows a normal epidermis and subcutaneous tissue with nearly total absence of the dermis Mental retardation is not a constant finding

Whether the cheilo-gnatho-palatoschisis seen in our patient is an associated but infrequent part of the syndrome or whether we are dealing with two independent congenital abnormalities is not known and in the present case no evidence for either of the hypotheses is available However one case of focal dermal hypoplasia associated with cleft *lip* has been described (5) The present patient has been referred to very briefly in a previous publication (1)

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CASE REPORT

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Case Reports

The two brothers were the only children of healthy unrelated parents. The parents had normal hair and abnormalities in the hair shaft could not be demonstrated by ordinary light microscopical investigation The serum copper and ceruloplasmin concentrations of both parents were normal

Case I

CÅT (210471/1467) (Fig 1) was the younger brother Pregnancy and delivery had been normal In particular no asphyxia was noticed. The birth weight was 2 840 g the length 48 cm There was icterus from the 3rd to the 7th day with maximal serum bilirubin value of 18 mg/100 ml When he was a few days old a peculiar pale yellow skin, woolly hair in tufts and pectus carinatum were noticed

At the age of 7 weeks he was admitted to the hospital because of failure to thrive and transitory jerks in the extremities. He was pale and thin with sparse, stubby kinky and fair hair and sparse eyebrows. The skin was dry but the nails normal The skull was compressed sidewards and the palate high arched The lower part of the sternum and the curvature were prominent. The tonus



Fig 1 Case I 3 / months old. The hair is sparse coarse and white

was elevated and the deep tendon reflexes hyperactive He was neither able to smile nor to fixate but the pupils reacted to light and the acoustico-palpebral reflex was present Most of the time he was quivering and had several attacks of hypotonicity combined with deviation of the eyes The attacks were partly controlled by phenobarbital and he began to thrive normally

At 4 months he was readmitted on account of increasingly frequent attacks Sometimes he was unconscious and akinetic for hours and at other times he had brief clonic convulsions The exterior was unchanged and the hair as earlier described. He showed no signs of psychomotor development At the age of almost 5 months he had no head control and could neither smile nor fixate He seemed unaware of his surroundings and did not react to light or sound A weak Moro- and grasp reflex remained The temperature was periodically subnormal (34–35°C) The head circumference had increased less than normal The prolonged attacks sometimes presented feeding difficulties but in spite of this he thrived well He died at the age of 5 months and 9 days from a pulmonary infection

Investigations

Ordinary blood and urine investigations were normal In particular it should be mentioned that the serum glutamin acid was normal and that arginosuccinic acid was not found in the urine The cerebrospinal fluid examined at the age of ? months was normal Echoencephalography revealed no dislocation of the central line EEG showed marked dysrythmia with a left side dominance of low frequencies. X-rays of the bones showed symmetrical metaphyseal spurrings, asymmetrical diaphyseal periostal thickenings and flaring of the anterior ends of the ribs The findings became most pronounced at 4 /₂ months Chromosomal analysis was normal Microscopy of the hair revealed pili torti (Fig. 2) trichorrhexis nodosa and monilethrix

A determination of serum copper and ceruloplasmin of a frozen blood sample taken at the age of 4 months revealed serum copper 13 μg/100 ml ceruloplasmin 0 05? g/l (the mean values of 3 healthy children at the same age were serum copper 145 μg/100 ml ceruloplasmin 0.4 g/l)

Autopsy

The organs were normal apart from the lungs which revealed bronchopneumonia a high division of aorta and origin of both a.a renales from the left a iliaca. Microscopy confirmed the changes of the lungs. The other organs the vessels and the bones, especially the costochondral junctions were normal

The neuropathological investigations will be reported in detail elsewhere (16) Only the essential results will be summarized

Gross examination revealed thickened leptomeninges, narrowed gyri and tortuous vessels on the brain surface

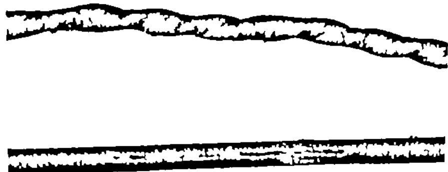


Fig 2 Scalp hair from case I showing pili torti (twisted hair) compared with a normal hair

Microscopical investigation A meningoencephalitis of a moderate degree was present, but not difficult to distinguish from the chronical alterations. The essential pathological changes were found in the hemispheres of the brain and the cerebellum, while the rest of the nervous system showed unimportant alterations. The most surprising findings were a nearly total destruction of the postnatally myelinated white matter with a relatively sharp border at the internal capsule. The myelin sheaths were relatively more profoundly affected than the corresponding axis cylinders. The cortex presented varying degrees of depopulation—not proportional to the severe alterations in the white substance.

In the cerebellum the molecular and the granular layers were atrophic. The population of the Purkinje cells was normal, but the individual Purkinje cells showed somal sprouts and grotesque branching of the dendritic tree. Torpedoes were not found. In the white matter moderate chronic degenerations and gliosis were seen.

In some of the cerebral arteries small focal processes were found beneath the intima and involving the internal elastic membrane.

Case II

UT (666/66) was the elder boy Pregnancy and delivery had been without complications. The birth weight was 3 200 g, the length 50 cm He did not present any problems until he was 1 month old He then began to quiver and to have jerks in his left arm. At the age of 2 months he was admitted to the hospital on account of diarrhoea and attacks of hypotonicity At that time he had almost no hair on his head. He developed the same clinical picture as his brother especially his temperature was constantly low (32 33°C). He died / years old from bronchopneumonia.

Investigations

Ordinary laboratory tests were normal. EEG showed an abnormal curve with poorly developed sleep pattern. Lumbar pneumoencephalography revealed slight ventricular dilatation X-rays of the cranium and the thorax were normal The hair was not examined

Autopsy

Bronchopneumonia was demonstrated The parenchyma of the right kidney was diminished and pelvis dilated All other extracranial organs were normal. Dura mater was thickened and the brain very small with narrowed gyri and widened sulci. There was a moderate hydrocephalus internus. Microscopical investigations were not performed

DISCUSSION

Two cases with a characteristic clinic of Kinky Hair Syndrome (KHS) are presented The diagnosis of the elder boy was determined retrospectively

Even when only a few days old the younger boy showed signs typical of the syndrome Only one patient among earlier described cases showed abnormal hair in the neonatal period (10) Another had hypothermia (8) and one was lethargic from birth (5) No other reported patients seem to have had characteristic symptoms until the 2nd or 3rd month

The low levels of serum copper and ceruloplasmin agree with the values found by Danks et al (4) It is their assumpton that the disease is caused by a copper deficiency The fact that the younger boy had signs of KHS even when he was a few days old would seem to show that he had developed the copper deficiency *in utero* This indicates that there could be other causes of copper deficiency than an intestinal malabsorption as postulated by Danks et al (4 6)

The abnormalities of the hair the bones and the vessels of the brain are in accordance with earlier descriptions The neuropathological findings in the cerebrum differ from other publications (1 5 9 10 11) and indicate that the primary lesion is located in the white matter (12)

Thus we have a typical case of KHS with pathological alterations of the ectodermal neuro-ectodermal and mesodermal tissue The changes of the hair bone and vessels might as pointed out by other authors (4 5 15) be explained by a copper deficiency How far the demyelinating process in the brain also is a consequence of a copper deficiency in our case is difficult to decide The copper concentration of the brain was normal (13) but it must be mentioned that the examination was performed on whole brain tissue without separation into cellular or subcellular fractions

Many factors in the copper metabolism in KHS have not yet been clarified Furthermore it would be interesting to know whether the mothers of KHS children have the normal increase of serum copper and ceruloplasmin during the last months of pregnancy (7)



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was elevated and the deep tendon reflexes hyperactive He was neither able to smile nor to fixate but the pupils reacted to light and the acoustico-palpebral reflex was present Most of the time he was quivering and had several attacks of hypotonicity combined with deviation of the eyes The attacks were partly controlled by phenobarbital and he began to thrive normally

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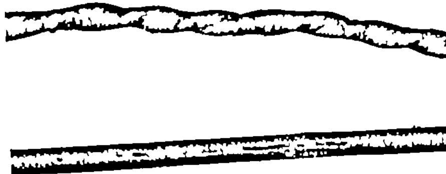


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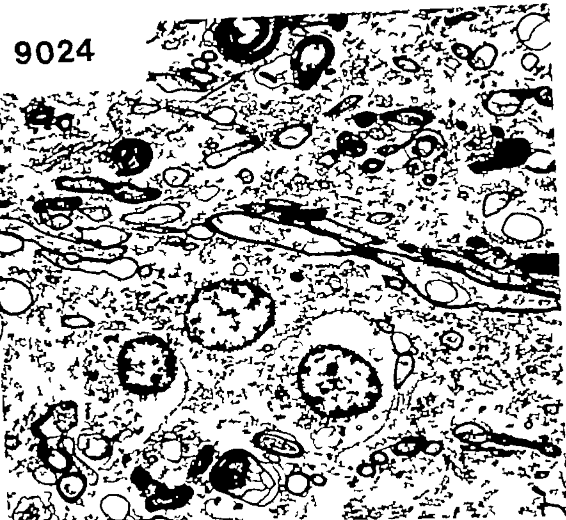


Fig 1 Electron micrograph of the white substance in the parietal region of our case with Menkes disease Note the degenerating myelin sheaths. Stain. Uranyl magnesium acetate and lead citrate, ×6000.

mesodermal and neuro-ectodermal structures and newer findings tend to include the entoderm as well (3)

From the beginning, interest and research concentrated on the field of neuropathology and clinical description—to try to find patho-anatomical correlates to clinical abnormalities. In the central nervous system the pathological changes are confined to the cerebral hemispheres and cerebellum. The prenatal myelination of the central nervous system and the peripheral nervous system is normal

In 1966 Aguilar et al (1) put forward the hypothesis that the *primary* point of attack in Menkes syndrome was both the cerebellar and the cerebral cortex together with *secondary* alterations in the white matter Contrary to this the Aarhus group (18) showed that the *primary* pathological process is found in the postnatally myelinating area of the cerebral hemispheres and that the cortical changes can be regarded as *secondary* on basis of the histopathological findings findings

This has been supported by biochemical

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The Editors have invited Dr Edith Reske Nielsen to give in connection with the case report of A M Møllekær a short review of the present knowledge of Menkes disease

MENKES DISEASE IS THE ETIOLOGY A COPPER TRANSPORT DEFECT?

In 1962 Menkes et al (10) described a new neurodegenerative disorder of early infancy which during recent years has become established as a well-defined syndrome Pedigree studies suggest a sex linked recessive inheritance but despite intensive clinical and biochemical research—especially since April 1972—the final clue has not been found in this intriguing and challenging disease which has now been reported in well over 25 cases

Menkes syndrome is a self-contained entity within the confusing area of neurodegenerative disorders of childhood and which is easy to diagnose clinically and serologically (2 10 11 19) The serum concentrations of copper and ceruloplasmin are low Apart from a characteristic clinical appearance—in which hypothermia especially seems to be a fairly regular feature—changes in bones and vessels are roentgenologically important signs Significant pathological anatomical abnormalities are found in both ectodermal

French et al (6) demonstrated a decreased intracellular oxidative metabolism in three cases of Menkes syndrome which may be due to copper deficiency He also showed that ultrastructural abnormalities of liver mitochondria in a case with Menkes disease disappeared completely after treatment with the chelate complex copper-calcium-ethylene diamine-tetraacetate

In animals with copper deficiency the clinical findings and the pathological anatomical changes are in agreement with those of Menkes syndrome in human cases (5 7 17) Of numerous disturbances in the intermediate metabolism the most significant—and also the earliest—dysfunction is a decreased activity of the cytochrome oxidase Abnormalities in the mitochondria and decreased synthesis of phospholipids are also found.

Lambs born after a fetal period characterized by deprivation of copper present symmetrical demyelinating processes in the cerebral hemispheres—but *not* in other parts of the central nervous system Human cases of Menkes disease present similar symmetrical pathological processes in the postnatally myelinating area of the white matter in the cerebral hemispheres—the prenatal myelination having proceeded as normal—presumably because of an adequate supply of copper as the albumen-bound fraction crossing the placental barrier (16)

On the basis of relatively few investigations performed in humans with Menkes syndrome it seems that the basic points to be remembered for the time being in this peculiar syndrome are

1 pathologic accumulations of copper in the intestinal mucosal cells
2. a normal copper content in whole-brain tissue and
3 low serum levels of copper and ceruloplasmin.

All three features indicate a postnatal disturbance in the transport mechanisms of copper ions—and may also explain the neuropathologic findings in early cases of Menkes disease (18)

At this stage it seems reasonable to conclude that children with Menkes syndrome have stores of not-available copper in the intestinal mucosal cells. It looks as if parenterally induced copper can be utilized by the tissues in Menkes syndrome and that the transport of copper in the body is disturbed —presumably in the mucosal cells but even the blood-brain barrier may be affected Bearing this in mind together with recent information from veterinary literature concerning the transport mechanisms of heavy metals by special proteins (4 12) an obvious and fascinating working hypothesis is that a similar protein may exist in man and that this vehicle may be absent or defective

Many important points remain for future research Additional neuropathological information from autopsies in cases with early death or from brain biopsies, is essential—including an attempt to localize copper at the cellular and subcellular level at the blood-brain barrier Further studies of the transport and localization of copper in the intestinal mucosal cells are also important Another important point may be to determine the serum concentrations of copper and ceruloplasmin In newborns immediately after birth and later—and in the mothers during pregnancy and after delivery In both cases treatment with parenteral copper may reveal important clues

Menkes syndrome is for good reasons the only usable name for this disorder of early childhood—perhaps until the final etiological trait is disclosed which presumably will place this syndrome among the inborn errors of metabolism The fact that the syndrome has been reported with increasing frequency during recent years seems to indicate that the number reported is out of proportion to the real frequency in pediatric materials—that it is much more common than earlier thought

Edith Reske-Nielsen

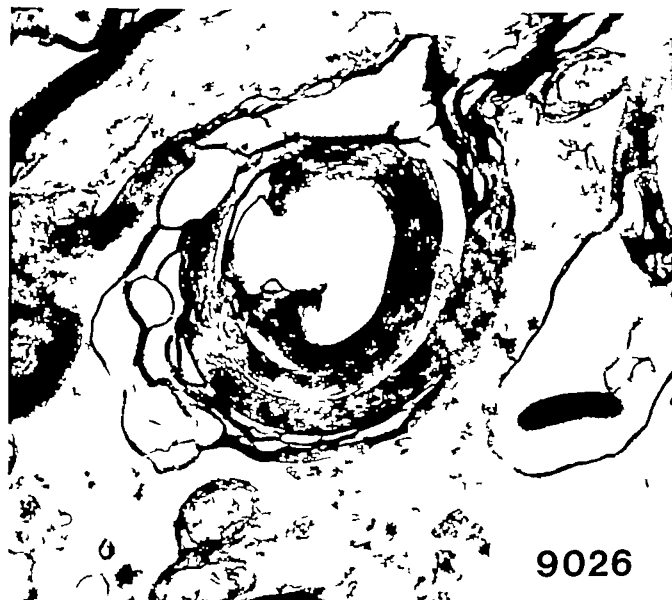


Fig 2 Higher magnification of Fig 1 (*arrow*) ×27 000

investigations (9) which demonstrated large amounts of esterified cholesterol in the white matter indicating—together with preliminary electron microscopical investigations (Figs 1 and 2)—the existence of a demyelinating process (14)

The copper content in whole brain tissue of the patient of Møllekær (11) was normal compared with two normal controls of the same age (13) but the cellular and subcellular distribution of copper—also in relation to the blood–brain barrier—has not yet been investigated (15)

The few available serological investigations all agree on low concentrations of copper and ceruloplasmin in serum Danks (2) was the first to direct attention to a defect in the absorption of copper but his investigations in 1973 showed that abnormally large amounts of copper can be demonstrated in the intestinal mucosa (4) Garnica (8) has confirmed this finding.

Intravenous administration of a copper–albumen complex raised serum copper and ceruloplasmin in a few patients to normal levels (3) Garnica could not confirm this after 10 days of treatment with cupriacetate in one case (8)

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CASE REPORT

NEONATAL AORTIC THROMBOSIS

A Possible Clinical Manifestation of Congenital Antithrombin III Deficiency

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ABSTRACT Bjarke, B., Herin, P. and Blombäck, M. (Department of Pediatrics, Karolinska Institutet, S:t Göran's Children's Hospital and Department of Blood Coagulation, Karolinska Sjukhuset, Stockholm, Sweden). Neonatal aortic thrombosis. Acta Paediat Scand 63: 297, 1974.—Two cases of neonatal aortic thrombosis are presented. Possible etiologies, as quoted in the literature, are discussed and the fact that the etiology seems to be unknown in at least half of the cases is stressed. The mothers of the two neonates presented had an antithrombin III (AT III) deficiency, which is associated with an increased tendency to thrombosis. One of the mothers had manifested such a tendency. The AT III deficiency as an etiological factor for the thrombus formation in the two babies is discussed.

KEY WORDS: Antithrombin III deficiency, neonatal aortic thrombosis, neonatal mortality

Occlusive thrombosis of the aorta is a rare disease in childhood and the diagnosis is often first noted at autopsy. Among children the risk of thrombo-embolism is greatest in the neonatal period (11, 20). No clear explanation for this tendency is known, nor has any etiological factor been found in more than half of the cases reported to date. The two cases described here may help to shed some light on the etiology of this intriguing disease.

CASE REPORTS

Case 1

First child of a 22-year old Swedish woman. Normal pregnancy during which the mother received no medication except mild diuretics. Normal spontaneous delivery. Birth weight 3100 g, length 50 cm. Pediatric examination before discharge from the maternity hospital at the age of 6 days revealed no signs of any disease.

The child was admitted to a pediatric hospital 1 day later with a 6–8 hour history of poor appetite, irritability and increasing respiratory derangement. Physical examination disclosed a critically ill, slightly dehydrated child with decreased muscular tone and general cyanosis, tachypnoea with pronounced inspiratory thorax movement and expiratory grunting; tachycardia but no heart murmur. The femoral pulse was not palpable and the axillary pulse was weak. Mild hepatomegaly.

Relevant laboratory findings showed a haemoglobin concentration of 19.4 g/100 ml and a haematocrit value of 66%. Blood gases revealed uncompensated metabolic acidosis. Electrolytes were normal. Blood cultures from the nose, throat, blood and cerebro-spinal fluid (C.S.F.) produced no growth. Chest X-ray revealed a slightly enlarged heart with a normal configuration, there were scattered infiltrates in both lung fields and even a slight amount of fluid in the right pleura.

The tentative diagnosis was septicaemia with bronchopneumonia and possible meningitis. The child died 18 hours after admission. No effort had been made to catheterize the umbilical vessels.

Post-mortem examination revealed that the heart's anatomy was normal with a physiologically open foramen ovale. The pulmonary artery, aortic arch, systemic and pulmonary veins were normal. The ductus arterio-

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disclosed pronounced cardiomegaly and some atelectasis in the left lung.

Coarctation of the aorta and incipient heart failure were suspected, and digitalis was administered. However the child's condition deteriorated. A bleeding tendency was noted, and repeated platelet counts and thrombotest values were pathological at 25000 per μl and 21% respectively. Urine formation ceased. An effort to catheterize the umbilical vein in order to perform spleno-cardiography failed as the ductus venosus could not be passed. Death occurred 12 hours after admission.

Post-mortem examination. A fresh thrombosis in the aorta, extending from the origin of the superior mesenteric artery down to the bifurcation, was found. Slight acute inflammation was noted in the aortic wall. The umbilical vessels were macroscopically normal. There were no signs of any congenital heart disease. The ductus arteriosus was wide open. Incipient gangrene was found in the proximal intestine. The right kidney was haemorrhagic but the left was unremarkable. The lungs were *hyperaemic* and the lower lobes showed suspected bleedings.

Family history. No history of thrombotic symptoms or any hereditary disease except allergy were known. The mother displayed no unusual symptoms during pregnancy. A coagulation investigation was performed on the mother about 1 month after delivery because of our previous experience with Case 1. The results are presented in Table 1.

METHODS

Antithrombin III (AT III) was assayed in three different ways.

1) Residual thrombin activity was assayed on plasma as substrate after incubation of patient plasma with thrombin according to the method of Abildgaard (2). The error of the method in our laboratory is ±2%. Normal range for 18 normal young men: 100 ±7.8% (M ± SD).

2) AT III biological activity was assayed using a synthetic tripeptide benzoyl-phenylalanine-valine-arginine-paranitroanilide as substrate for determining residual thrombin activity after incubation of patient plasma with known amount of thrombin (28). The error of the method in our laboratory is ±6%. Normal range for 18 normal young men: 100 ±12.8% (M±2 SD).

3) The quantitative radial immunodiffusion technique was used as described by Fagerhol & Abildgaard (10). The error of the method in our laboratory is ±6%. Normal range for 18 normal young men: 100 ±13.[illegible]% (M±SD).

DISCUSSION

Intravascular thrombosis rarely occurs in childhood (11, 18, 19). Venous thrombosis is more frequent than arterial thrombosis. Thrombosis in the renal and adrenal veins and intracranial sinuses during the neonatal period are frequently associated with prolonged labour, maternal diabetes and diseases producing high haematocrit values (22, 24, 26, 27, 29).

The etiology of arterial thrombo-embolism varies and is unknown in many cases. Infection, either local or general, has been found in several cases (11, 17, 19, 25). Particular attention has been devoted to umbilical vessel infections (21, 25). Post-partum haemodynamic changes, especially closure of the ductus and even atrophy of the umbilical arteries, are believed to be etiological factors in a number of cases (11, 18, 23, 27). Several authors have attributed etiological significance (13, 19, 20) to either degenerative or inflammatory changes in the aortic wall. Arrhythmias and congenital heart disease, especially involving cyanosis with polyglobulia, sometimes give rise to thrombus formation and embolism. It is also well known that catheterization of the umbilical vessels, especially the umbilical arteries, may induce formation of thrombi. It appears as if any artery may be the site of thrombi. However, obstructions are more likely to occur in peripheral vessels, and obstruction of the aorta is very rare indeed. A total of about 30 children with aortic thrombosis have been described. Reviews of reported cases have been published (11, 18, 19, 25).

None of the above-mentioned etiological factors were probably of any significance in our cases. To our knowledge neither thorough coagulation studies were performed in cases previously reported, nor has there been any information about thrombotic predisposition among parents or relatives.

The mothers of the two children described by us had low levels of antithrombin III (AT III) as determined by three different methods. AT III or progressive antithrombin is responsible for most of the anticoagulant action of antithrombin. This antithrombin has a (at least) 3-fold mode of action (1)

Table 1 *Results of coagulation investigation*

Analyses	Mother of patient 1*					Mother of patient 2	
	Aug. 1970	Sept 1970	Oct 1970	Feb 1971	March 1973	Nov 1972	March 1973
Platelets per ul	238 000	–	212000			224000	
Bleeding time Ivy	3 57"	–				–	
Recalcification time	N	–	N			–	
Partial thromboplastin time	–	–	N			–	
Activated thromboplastin time	–	–	–			N	
Prothrombin consumption	N	–	N			–	
Russel Viper Venom time	N	–	N			–	
One-stage prothrombin time	N	–	N			–	
Thrombin time	–	–	N			N	
Reptilase time	–	N	–			N	
Factor VIII % of n	88	–	–			107	
Factor IX % of n	–	–	74			–	
Factor V % of n	–	–	117			112	
P × P % of n.	–	–	99			–	
Normotest % of n	–	74	–			96	
Prothrombin % of n.	88	–	127			–	
Fibrinogen g/100 ml	0 27	–	0 30			0 29	
Factor XIII	N	–	N			–	
Alfa$_2$-macroglobulin % of n.	–	–	–			98	
Antithrombin III							
Biol. coag. activity (2) % of n.	–	43	–	50	55	–	40
Biol amidolyt activ (28) % of n.	–	–	–		60		70
Immuno assay (10) % of n	–	de-creased	65	51	50	53	48

Earlier partly reported (14)

sus showed signs of physiological closure but could be probed In the left atrial appendix there was a small thrombus protruding into the atrium In the abdominal aorta slightly below the take-off of the superior mesenteric artery there was a totally occlusive thrombus extending down the aorta into the iliac arteries The thrombus was of recent origin and loosely attached to the wall. Microscopic examination disclosed no pathological changes in the aortic wall which could explain the thrombus formation Microscopy of the lungs disclosed widely scattered interstitial and intra-alveolar haemorrhage No thrombosis could be found in the renal veins or the venae cavae Microscopic examination showed haemorrhagic infarction of the kidneys No sign of inflammation was detected when the umbilical vessels were examined microscopically

Family history Two years prior to the birth of the child described above the mother delivered a still-born baby after an uneventful 8-month pregnancy Post-mortem examination of this female child showed advanced maceration but no malformations could be found The heart and the great vessels were reported to be normal However no additional investigation of the circulatory system was probably performed. Two weeks after delivery of this still-born child, the mother developed a right femoral vein thrombosis with pulmonary embolism Right femoral vein thrombectomy was performed Also after the second child (Case 1) the mother developed a deep femoral vein thrombosis which was treated conservatively In view of the mother s history of repeated thrombo-embolic episodes and the findings in the child described it was arranged for the mother to have a thorough coagulation analysis. The results are summarized in Table I

Case 2

First child of a 27 year-old Swedish primigravida. There was an uneventful 40-week pregnancy during which the mother received no medication Normal spontaneous delivery Birth weight 3180 g, length 51 cm Good initial activity

On the fourth day of life progressive feeding problems colour impairment and respiratory distress developed The child was admitted to a pediatric hospital on these grounds at the age of 5 days and was there found to be in poor condition with tachypnoea tachy-

disclosed pronounced cardiomegaly and some atelectasis in the left lung.

Coarctation of the aorta and incipient heart failure were suspected, and digitalis was administered. However the child's condition deteriorated. A bleeding tendency was noted, and repeated platelet counts and thrombotest values were pathological at 25 000 per µl and 21% respectively Urine formation ceased. An effort to catheterize the umbilical vein in order to perform angio-cardiography failed as the ductus venosus could not be passed. Death occurred 12 hours after admission.

Post-mortem examination A fresh thrombosis in the aorta, extending from the origin of the superior mesenteric artery down to the bifurcation, was found Slight acute inflammation was noted in the aortic wall The umbilical vessels were macroscopically normal There were no signs of any congenital heart disease The ductus arteriosus was wide open Incipient gangrene was found in the proximal intestine The right kidney was haemorrhagic but the left was unremarkable The lungs were hyperaemic and the lower lobes showed suspected bleedings.

Family history No history of thrombotic symptoms or any hereditary disease except allergy were known The mother displayed no unusual symptoms during pregnancy A coagulation investigation was performed on the mother about 1 month after delivery because of our previous experience with Case 1 The results are presented in Table 1

METHODS

Antithrombin III (AT III) was assayed in three different ways

1) Residual thrombin activity was assayed on plasma as substrate after incubation of patient plasma with thrombin according to the method of Abildgaard (2) The error of the method in our laboratory is ±2% Normal range for 18 normal young men. 100 ±7.8% (M±2 SD).

2) AT III biological activity was assayed using a synthetic tripeptide benzoyl-phenylalanine-valine arginine-paranitroanilide as substrate for determining residual thrombin activity after incubation of patient plasma with known amount of thrombin (28). The error of the method in our laboratory is ±6% Normal range for 18 normal young men 100 ±12.8% (M±2 SD)

3) The quantitative radial immunodiffusion technique was used as described by Fagerhol & Abildgaard (16) The error of the method in our laboratory is ±6% Normal range for 18 normal young men 100 [illegible] % (M±SD)

DISCUSSION

Intravascular thrombosis rarely occurs in childhood (11 18 19) Venous thrombosis is more frequent than arterial thrombosis Thrombosis in the renal and adrenal veins and intracranial sinuses during the neonatal period are frequently associated with prolonged labour maternal diabetes and diseases producing high haematocrit values (22, 24 26 27 29)

The etiology of arterial thrombo-embolism varies and is unknown in many cases Infection either local or general has been found in several cases (11 17 19 25) Particular attention has been devoted to umbilical vessel infections (21 25) Post-partum haemodynamic changes especially closure of the ductus and even atrophy of the umbilical arteries are believed to be etiological factors in a number of cases (11 18 23 27) Several authors have attributed etiological significance (13 19 20) to either degenerative or inflammatory changes in the aortic wall Arrhythmias and congenital heart disease especially involving cyanosis with polyglobuli sometimes give rise to thrombus formation and embolism It is also well known that catheterization of the umbilical vessels especially the umbilical arteries may induce formation of thrombi It appears as if any artery may be the site of thrombi. However obstructions are more likely to occur in peripheral vessels and obstruction of the aorta is very rare indeed A total of about 30 children with aortic thrombosis have been described Reviews of reported cases have been published (11 18 19 25)

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It inhibits the action of thrombin by forming an inactive complex it inactivates coagulation factor Xa (1) and it prevents the platelet aggregation induced by thrombin It should also serve as a heparin co factor by virtue of its inhibition of thrombin–fibrinogen interaction (1) a view which is not in agreement with the findings of many investigators (1 6) however

Low levels of AT III and decreased anticoagulant activity measured as antithrombin activity or by immunochemical means have been associated with increased intravascular coagulation and with an increased incidence of thrombo-embolic episodes Thus a decreased AT III level is found in patients with severe liver disease (3 12) in disseminated intravascular coagulation (3) in women taking oral contraceptives (9 10) and during acute thrombo-embolic episodes (3) AT III levels are also known to be depressed in women during the second and third trimesters of pregnancy and during the first days after delivery (5 16) None of the mothers described by us were taking any oral contraceptives at the time of the coagulation studies Nor did they display any signs of liver disease

The best examples of a link between a low antithrombin level and thrombo-embolism are found in patients with hereditary deficiencies (3 7 8) Several members of the family described by Egeberg in 1965 displayed a definitely pathological predisposition for thrombosis and afflicted persons had low AT III levels (8) A 50% AT III reduction greatly increased the risk of venous thrombosis and pulmonary emboli AT III deficiency was found both in males and in females and this deficiency was transferred to children of both sexes Thus the defect appeared to be inherited as a dominant autosomal trait

It is highly likely that the two mothers in this study represented cases of congenital AT III deficiency The incidence of this disorder is unknown In view of the above it is tempting to regard the thrombus formation in our two children as a manifestation of congenital AT III deficiency It is true that the thrombi in the families described by Egeberg (8) von Kaulla et al (15) and Dejanov et al (7) were of venous origin However it may be that a moderate deficiency in AT III may not manifest itself until later in life and that involvement of the arterial system is unlikely as long as homeostasis is not severely deranged Very low levels may become manifest in the neonatal period then possibly involving arterial vessels A coagulation examination should be performed with special reference to the AT III level in the parents of children with thrombosis and if possible in the children themselves

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ADDENDUM

The mother of patient no 2 recently (Nov 1973) gave birth to twins. Both had abnormally low antithrombin levels at birth. One died, severely brain damaged au topsy showing a big venous thrombosis in the inferior caval vein and right atrium of the heart. The other baby who is still living (5 weeks old) is treated with plasma (antithrombin) and heparin.

Acta Pædiat Scand 63 302–306 1974

CASE REPORT

PLASMA INSULIN AND BLOOD GLUCOSE DURING LONG TERM TREATMENT WITH DIAZOXIDE FOR INFANT HYPOGLYCEMIA

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ABSTRACT Victorin L. H. and Thorell, J (Department of Paediatrics, University of Göteborg Göteborg and the Isotope Laboratory Lund University at the General Hospital, Malmö Sweden) Plasma insulin and blood glucose during long-term treatment with diazoxide for infant hypoglycemia. Acta Paediat Scand, 63 302, 1974.—Hypoglycemia of varying etiology is a major therapeutic problem in infancy and childhood It has been shown that diazoxide may increase blood glucose presumably mainly by prevention of excess insulin release from the pancreas. A case is reported where diazoxide has been used for more than three years in a boy with neurological symptoms due to severe idiopathic hypoglycemia with leucine intolerance where dietary treatment had proved insufficient. A profound improvement in blood glucose level and suppression of pathologic insulin response to both glucose and leucine loads was noted with a diazoxide dose of 15 mg/kg/day which, however had to be abandoned due to side effects. Over a period of months without diazoxide insulin responses to glucose and leucine loads progressively increased with recurrence of clinical symtoms. Over the last three years a dose of 5 mg/kg/day has proved effective in keeping clinical symptoms down without side effects During these years a marked improvement has taken place in neurological mental and physical development. It is concluded that when due consideration is given to known side effects diazoxide is a valuable adjuvant for long-term treatment of infantile hypoglycemia

KEY WORDS: Hypoglycemia diazoxide insulin, glucose

Hypoglycemic disease starting during the first 6 months of life is associated with a high risk of persistent mental and neurological sequelae (6 11 16 18) The disease is often difficult to classify etiologically and most cases have been termed idiopathic so implying symptomatic treatment regimes Also even in cases where a specific diagnosis can be made such as leucine intolerance a non specific mode of therapy to increase blood sugar is often necessary Thus it was of great interest when Drash & Wolff in 1964 (7) reported favorable results from treatment of severe hypoglycemia with diazoxide

Diazoxide (3 methyl 7-chloro-1,2 4-benzothiadiazine) belongs to the group of benzothiadiazines used in the management of hypertension Given orally it is a very weak antihypertensive agent and has as a side effect the creation of a pronounced hyperglycemia The mechanism behind the increase in blood sugar is complex Of greatest importance is probably a direct inhibition of insulin release from the pancreatic beta-cells (12 19) There is also evidence in favour of both an indirect adrenergic mediated hyperglycemia and a direct adrenergic effect on the liver (12 15 19)

Diazoxide therapy has also been tried in in

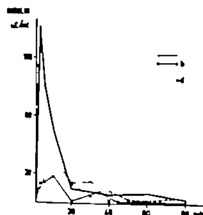


Fig. 1. Insulin response to intravenous glucose load in a 30% solution 0.5 g/kg bw. (*a*) before treatment with diazoxide. (*b*) 3 weeks on D 15 mg/kg bw. (*c*) 1 week after withdrawal of D. (*d*) 2 months after withdrawal of D.

fancy and childhood although with a varying degree of success (1, 2, 8, 10, 21, 22, 23). This communication describes the results of long-term diazoxide[1] therapy in a boy with symptoms of hypoglycemia from the first month of life who has been treated for more than 3 years.

CASE REPORT AND RESULTS

The patient is the second son of healthy unrelated parents. The brother has shown no sign of hypoglycemic disease. No remarkable symptoms were noted during the immediate postnatal period. Birth weight 3 520 g. At 4 weeks of age the patient exhibited jitteriness. This symptom progressed to such a degree that the boy was hospitalized at 4 months. EEG was normal, fasting blood sugar 36 mg/100 ml. Insulin load had to be discontinued due to convulsions. A diet rich in carbohydrates with leucine restriction was instituted.

During the second part of the first year cerebral symptoms increased with the development of grand-mal seizures, progressive mental and motor retardation, and antiepileptic treatment was started. EEG at 12 months showed bilateral synchronous epileptic activity. Echo-encephalography showed a dilated ventricular system verified by air encephalography with central and cortical atrophy.

Psychological development according to Griffith's developmental scale at 9 months was somewhat delayed but within normal limits. Six months later definite developmental retardation had occurred; at a biological age of 17 months, the developmental age was 10–1 months.

At 15 months, when the patient was first seen by us, glucose load (0.5 g/kg bodyweight of a 30% solution intravenously) induced an extremely high peak in plasma insulin level as determined with a radioimmunoassay** (Figs. 1, 3); leucine load (150 mg/kg bodyweight orally) had to be discontinued after a few minutes due to convulsions. With tolbutamide loading, blood glucose fell to 10 mg/100 ml at 10 minutes. The pathologic activity on EEG had progressed further.

Diazoxide treatment 15 mg/kg/day was started at 16 months. After one day the patient was more alert without convulsions. A normalization of insulin response to glucose and leucine loading (Figs. 1, 2), decreased glucose disappearance rate and normalization of fasting blood glucose was noted (Fig. 3).

Diazoxide was discontinued after 6 weeks due to ketosis and hypertrichosis. One week later insulin response to glucose and leucine loads were again elevated (Figs. 1, 2, 3). Repeated glucose and leucine loads created a successively increasing insulin response. After 4 weeks the hypertrichosis had disappeared and the EEG was normal. Over this time the anticonvulsive therapy was slowly discontinued without any noticeable clinical attacks.

Convulsive episodes associated with an anomalous EEG reappeared 6 months after cessation of diazoxide. At this time both glucose and leucine loads produced high insulin levels. Diazoxide was reinstituted and maintained at a dose of 5 mg/kg/day. The patient has since then been virtually asymptomatic with normal fasting blood sugar (Fig. 3) with the exception of some episodes of somnolence and twitching apparently associated with increased leucine consumption.

Despite continued rather high insulin responses, especially to leucine loads (Fig. 3), the EEG and echo-encephalograms have normalized. Psychomotor development is in accordance with actual age. Side effects have not been seen during the second term of treatment lasting for more than 3 years. No signs of edema, tachycardia or postural hypotension have been noted. White cell blood count, thrombocytes, serum electrolytes, FFA, triglycerides and blood uric acid are all within normal limits. No hirsutism or other dermatological pathology have reappeared. Skeletal maturation is normal for age.

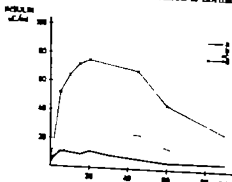


Fig. 2. Insulin response to oral leucine load 150 mg/kg bw. (*a*) after 3 weeks on diazoxide 15 mg/kg bw. (*b*) 2 days after withdrawal of D. (*c*) 2 weeks after withdrawal of D. (*d*) 2 months after withdrawal of D.

[1] Supplied as PROGLICEM by Schering Corporation, USA.

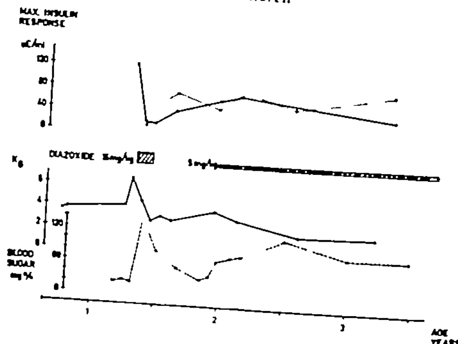


Fig 3 Longitudinal changes w... and without diazoxide therapy ... the top of the figure the full li... represents insulin response to gl... cose loads the dotted line the re... sponse to leucine loads. Gluco... disappearance rate is represented b... the K_g values

DISCUSSION

This case illustrates the development of serious neurological abnormalities in association with a hypoglycemic syndrome during the first years of life Despite the institution of dietary treatment the disease showed a progressive course The negative results of diagnostic and therapeutic measures directed against a supposed coexisting primary neurological disorder and the beneficial course of the condition with and without diazoxide therapy support the view that the cerebral symptoms were caused by hypoglycemia

One main diagnostic problem before diazoxide treatment was instituted in this case was to differ between insuloma and idiopathic hypoglycemia of the new born There are no criteria that infallibly exclude an insuloma but the early start of symptoms low basal plasma insulin levels and favourable result of a therapeutic trial with diazoxide were at the time considered strong evidence against this diagnosis and instead speaking in favour of a functional hypoglycemia A just recently described case by Baerentsen (1) may question the validity of these criteria

The fact that diazoxide the main effect of which probably is to inhibit the release of pancreatic insulin is reported beneficial also in cases of idiopathic hypoglycemia indicates that an excessive insulin secretion may play a role in this condition The group of conditions with proven hyperinsulinemia in childhood involves besides children with fetal erythroblastosis or diabetic foetopathia rare conditions such as insulinproducing insuloma so called β-cell nesidoblastosis and probably includes the leucine sensitive hypoglycemia as well (1 9 14 17 20 23 25) But the overwhelming majority of children with hypoglycemia does not belong to any of these categories However it is very difficult to define whether a hypoglycemic state is associated with elevated insulin levels or not which is demonstrated by cases with proven β-cell insuloma and hypoglycemia in which it was not possible to establish any hyperinsulinemia and still the hypoglycemia was cured by surgical removal of the tumour (1 9 13) These circumstances indicate that our possibilities of identifying a relative hyperinsulinemia still are inadequate and that excessive insulin secretion may actually be more common than is generally believed

The case presented in this communication clearly illustrates the effect of diazoxide to inhibit the release of insulin The high pretreatment insulin response was diminished by institution of treatment in parallel with marked clinical improvement In the subse

Table 1 *Side effects of oral diazoxide therapy*
Total number of cases. 103

Side effects	No. of Cases
1 Hirsutism	25
2. Gastrointestinal intolerance (anorexia, vomiting, pain)	16
3. Edema	17
4 Tachycardia (supraventricular)	9
5. Postural hypotension	
6. Hematological (neutropenia, eosinophilia, thrombocytopenia, anemia lymphocytosis)	8
7 Dermatological (maculopapular rash, malar flush, photosensitivity)	4
8. Hyperuricemia (transient, asymptomatic)	11
9 Immunoglobulin decrease	4
10 Lymphadenitis	1
11 Gingival hyperplasia	1
12. Muscle wasting	1

Compiled by Howard N Schwartz, M D Clinical Pharmacology Schering Corporation Bloomfield N.J

quent course these relations showed a marked consistency: diminished doses of diazoxide were followed by increased insulin responses and impaired clinical status and vice versa

The initial dose of diazoxide 15 mg/kg/day had a strong and prompt clinical effect with disappearance of hypoglycemic symptoms and normalization of blood sugar glucose disappearance rate and insulin response to both leucine and glucose loads Therapy was discontinued after 6 weeks due to the development of pronounced hypertrichosis and ketosis. The child was otherwise clinically well

Side effects with diazoxide treatment are not rare (Table 1) They are all described as reversible on discontinuation of therapy (3 8) which is also illustrated by the present case where both hypertrichosis and ketosis had disappeared after a few weeks. The case also shows the dose dependency of the side effects. the lower dose of 5 mg/kg/day has been well tolerated for over 3 years during the second period of treatment.

Of special interest is the very slow deterioration during the 6-month period with only dietary treatment which followed the first treatment with high-dose diazoxide Fasting blood sugar decreased in association with a slight increase in glucose disappearance rate This slowly fading beneficial effect of diazoxide also reflected in maximal insulin response to glucose and leucine loads has been described earlier in adults with insuloma (4 22) but not in small children with uncomplicated leucine intolerance or idiopathic hypoglycemia. Still the very favorable clinical results obtained with the smaller dose of 5 mg/kg/day given after recurrence of symptoms has prompted prolonged continuous diazoxide treatment.

Even though the leucine intolerance is still marked as reflected in insulin response during loads the patient has gradually been catching up with his biological age in both mental and motor skills The case described shows that when due consideration is given to known side effects diazoxide is a valuable adjuvant for long term treatment of infantile hypoglycemia.

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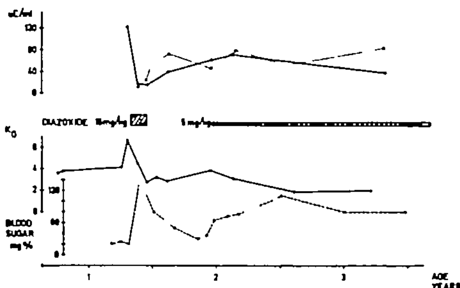


Fig 3 Longitudinal changes with and without diazoxide therapy At the top of the figure the full line represents insulin response to glucose loads, the dotted line the response to leucine loads Glucose disappearance rate is represented by the k_G values

DISCUSSION

This case illustrates the development of serious neurological abnormalities in association with a hypoglycemic syndrome during the first years of life Despite the institution of dietary treatment the disease showed a progressive course The negative results of diagnostic and therapeutic measures directed against a supposed coexisting primary neurological disorder and the beneficial course of the condition with and without diazoxide therapy support the view that the cerebral symptoms were caused by hypoglycemia

One main diagnostic problem before diazoxide treatment was instituted in this case was to differ between insuloma and idiopathic hypoglycemia of the new born There are no criteria that infallibly exclude an insuloma but the early start of symptoms low basal plasma insulin levels and favourable result of a therapeutic trial with diazoxide were at the time considered strong evidence against this diagnosis and instead speaking in favour of a functional hypoglycemia A just recently described case by Baerentsen (1) may question the validity of these criteria

The fact that diazoxide the main effect of which probably is to inhibit the release of pancreatic insulin is reported beneficial also in cases of idiopathic hypoglycemia indicates that an excessive insulin secretion may play a role in this condition The group of conditions with proven hyperinsulinemia in childhood involves besides children with fetal erythroblastosis or diabetic foetopathia rare conditions such as insulinproducing insuloma so called β-cell nesidoblastosis and probably includes the leucine-sensitive hypoglycemia as well (1 9 14 17 20 23 25) But the overwhelming majority of children with hypoglycemia does not belong to any of these categories However it is very difficult to define whether a hypoglycemic state is associated with elevated insulin levels or not which is demonstrated by cases with proven β-cell insuloma and hypoglycemia in which it was not possible to establish any hyperinsulinemia and still the hypoglycemia was cured by surgical removal of the tumour (1 9 13) These circumstances indicate that our possibilities of identifying a relative hyperinsulinemia still are inadequate and that excessive insulin secretion may actually be more common than is generally believed

The case presented in this communication clearly illustrates the effect of diazoxide to inhibit the release of insulin The high pretreatment insulin response was diminished by institution of treatment in parallel with marked clinical improvement In the subse-

Table 1 *Side effects of oral diazoxide therapy*
Total number of cases: 103

Side effects	No. of Cases
1 Hirsutism	25
2 Gastrointestinal intolerance (anorexia, vomiting, pain)	16
3. Edema	17
4 Tachycardia (supraventricular)	9
5. Postural hypotension	2
6. Hematological (neutropenia eosinophilia, thrombocytopenia, anemia, lymphocytosis)	8
7 Dermatological (maculopapular rash malar flush, photosensitivity)	4
8 Hyperuricemia (transient, asymptomatic)	11
9 Immunoglobulin decrease	4
10. Lymphadenitis	1
11 Gingival hyperplasia	1
12 Muscle wasting	1

Compiled by Howard N Schwartz, M D Clinical Pharmacology Schering Corporation, Bloomfield, N.J

quent course these relations showed a marked consistency: diminished doses of diazoxide were followed by increased insulin responses and impaired clinical status and vice versa.

The initial dose of diazoxide, 15 mg/kg/day had a strong and prompt clinical effect with disappearance of hypoglycemic symptoms and normalization of blood sugar glucose disappearance rate and insulin response to both leucine and glucose loads Therapy was discontinued after 6 weeks due to the development of pronounced hypertrichosis and ketosis. The child was otherwise clinically well

Side effects with diazoxide treatment are not rare (Table 1) They are all described as reversible on discontinuation of therapy (3 8) which is also illustrated by the present case where both hypertrichosis and ketosis had disappeared after a few weeks. The case also shows the dose dependency of the side effects: the lower dose of 5 mg/kg/day has been well tolerated for over 3 years during the second period of treatment.

Of special interest is the very slow deterioration during the 6-month period with only dietary treatment which followed the first treatment with high-dose diazoxide Fasting blood sugar decreased in association with a slight increase in glucose disappearance rate This slowly fading beneficial effect of diazoxide also reflected in maximal insulin response to glucose and leucine loads has been described earlier in adults with insuloma (4 22) but not in small children with uncomplicated leucine intolerance or idiopathic hypoglycemia. Still the very favorable clinical results obtained with the smaller dose of 5 mg/kg/day given after recurrence of symptoms has prompted prolonged continuous diazoxide treatment.

Even though the leucine intolerance is still marked as reflected in insulin response during loads the patient has gradually been catching up with his biological age in both mental and motor skills The case described shows that when due consideration is given to known side effects diazoxide is a valuable adjuvant for long term treatment of infantile hypoglycemia.

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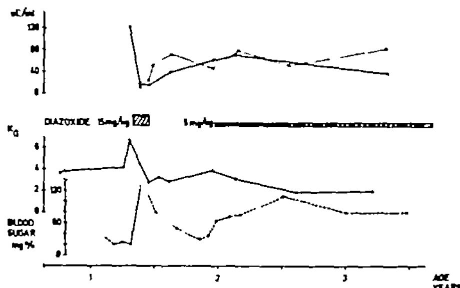


Fig 3 Longitudinal changes wit and without diazoxide therapy A the top of the figure the full lo represents insulin response to glu cose loads the dotted line the re sponse to leucine loads. Glucose disappearance rate is represented by the K_G values

DISCUSSION

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

SCANDINAVIAN ASSOCIATION OF PAEDIATRIC SURGEONS
SPANISH SCANDINAVIAN MEETING

Meeting May 25–28 1972

Panel I Hirschsprung s Disease

F Contreras A. Segura & M Claver (Madrid Spain): *The morphopathological study in Hirschsprung s disease*

Simple morphological data (dilatation hypertrophy presence or absence of neurons etc) and more recent histochemical methods have enabled a very satisfactory management of patients with Hirschsprung s disease to be achieved. However a review of our own material (91 biopsies and 61 surgical specimens) prompts us to comment on the possibilities and limitations of the diagnosis and surgical management.

The junction of aganglionic and ganglionic segments presents variable morphological and histochemical patterns and there is actually not enough information for positive diagnosis or fixing the limit of resection on this basis if the specimen is taken from the transitional zone True hypoganglionism and disturbed maturation of intramural plexa may clinically simulate Hirschsprung s disease Incorrect surgical treatment may be instigated by a false positive or negative histological diagnosis. No definitive histochemical data able to aid the differential diagnosis are available at present.

Histochemical studies (mitochondrial enzymes fluorescence methods for adrenergic fibres and acetylcholinesterase activity) have been valuable to the modern concepts of Hirschsprung s disease they will probably also be greatly helpful in establishing the different patterns at the junctional zone The demonstration of acetylcholinesterase activity has allowed the routine use of suction biopsies in the diagnosis of Hirschsprung s disease However in order to fix the limit of intestinal resection some conceptual and technical limitations set forth in this paper should be borne in mind

O Knutrud (Oslo Norway): *The surgical management of total colonic and partial small intestinal aganglionosis*

Eight cases of small intestinal aganglionosis including three siblings were seen at the Paediatric Surgical Department in Oslo 1959–72 during which period 106 patients with Hirschsprung s disease were treated

There are no definitive preoperative diagnostic clues to this syndrome but certain points rather suggestive of the condition are elicited by a review of our radiographic material Plain abdominal X-rays usually show low-type small intestinal obstruction such as that seen in ileal atresia, but careful examination often revealed a small amount of gas in the rectum A "used colon slightly smaller than normal was usually seen on barium enema, quite distinct from the classical microcolon in small intestinal or

meconium ileus An attempt should also be made to fill the terminal ileus which may appear greatly narrowed in aganglionosis of the small intestine Moreover poor evacuation of contrast material is usually observed in a follow-up X ray 24 hours later

There were 7 primary survivors in our series of 8 cases which presented aganglionosis of the whole small intestine thus presenting a hopeless situation The patient lost was one of the three siblings the other two survived at first and were subjected to ileoproctostomy with 4 and 1½ years survival after the definitive surgical intervention Both died later of vomit aspiration The remaining 5 patients are alive and well

Of the 5 late survivors only one has an intact colon In 2 other patients the colon was not removed at the time of the radical procedure but this had to be done later on when abdominal distension and colonic infection ensued resulting in perirectal and pericolonic abscesses

We consider it important to stress that the colon should be removed at the time of the definitive procedure

I Louhimo (Helsinki Finland) *Definitive treatment of Hirschsprung s disease with special reference to the Duhamel Sulamaa operation*

At the Children s Hospital of the University of Helsinki 141 definitive operations for Hirschsprung s disease have been performed trying out all methods commonly used Swenson 28 Rehbein 8 Soave 5 Duhamel 100 All these may produce excellent results but in our experience Duhamel s procedure with its modification has proved safest During the last 5 years in Duhamel s operation a special clamp designed by Sulamaa has been used (*Ann Chir Infant* 9 63 1968) which aims to prevent septum formation and to eliminate the necessity of rectal closure or of anastomosis deep in the pelvis The results of the first 23 Duhamel-Sulamaa operations have now been analysed

The series comprised 18 boys and 5 girls, aged at the time of the operation less than 1 year 12 cases 1–2 years 6 and over 2 years 5 There were no deaths nor any complications due to a leaking stump It is obvious however that septum formation, although less frequent than after the original Duhamel procedure has not been completely eliminated Secondary crushing of septum was necessary in 6 patients two of them also suffering from stenosis of the colorectal anastomosis One patient required secondary sphincterotomy for constipation Postoperative soiling was seen in 5 patients but this is associated with septum formation plus fecal impaction and is likely to disappear now that the septum has been crushed Most of the complications were associated with premature removal of clamp (earlier than 7 days)

It is concluded that the Duhamel Sulamaa operation is safe and easy to perform Scrupulous care in placing the clamp and in dealing with the internal sphincter will probably improve the results The operation can be recommended for further use

J Garrido-Lestache V Rollan & J M Ollero (Madrid Spain) *Hirschsprung s disease*

The author s personal series of 56 cases is from a period of 13 years (Jan 1959 to Jan 1972) and corresponds to 5 000 histories of pediatric surgical patients 0–14 years old The series consists of 38 males and 18 females 18 of the patients were younger than 3 months the rest were older (oldest patient 12 years)

In addition to usual clinical findings (delayed meconium expulsion pseudo-obstructive episodes constipation diarrhoea, etc.) the diagnostic criteria included radiological demonstration of a constricted segment Rectal biopsy was only performed in 12 ambiguous

cases: 7 of them were definitive of Hirschsprung s disease while 5 gave evidence of normal intramural plexa

The aganglionic segment was classical in extent (recto-colic) in 38 patients and short in 12. No subtotal colonic aganglionosis was encountered although 2 out of 4 cases with long colic aganglionic segments showed aganglionosis in the terminal third of the ileum.

An emergency colostomy was needed owing to poor general condition in 9 of the 18 patients younger than 3 months the mortality was 55% among these cases and as high as 75% among 4 infants of this age group with long aganglionic segments. Among 4 other cases of this group with a good general condition Duhamel repair resulted in one death

In the older age group the Duhamel procedure was applied in 36 children, with only two fatalities (both in 1960) due to operative technique (mortality 5.5%)

J Tovar (Madrid, Spain): *The management of Hirschsprung s disease in newborn and infants*

In a series of 118 cases of Hirschsprung s disease from 1966–72, the mortality in the group of 38 patients younger than 3 months was 38 6% and among the 80 older patients it was 2.5%

Monereo s finding is endorsed that small bowel participation in the disease through caeco-ileal reflux is mainly due to incompetence of the ileocaecal valve and contributes in producing diarrhoea enterocolitis and ultimately a high mortality rate Such reflux was radiologically established in 22 of the 118 patients 20 of them younger than 3 months

Eight cases with a short aganglionosis were managed by simple nursing up to the radical operation. A colostomy was performed in 15 cases with a long colic aganglionism as a first measure and in another 5 cases at different phases of the treatment.

Ileo-caeco-plication (Monereo 1964) is proposed for cases showing signs of caeco-ileal reflux that is considerable dilatation of the smaller bowel without megacolon and diarrhoea This operation very simply renders the ileocaecal valve competent avoiding reflux and allowing nursing up to the time of pull-through It is better tolerated than colostomy The procedure was applied in 17 cases with 3 deaths but of which only one can be related to the procedure

M Kabel (Copenhagen Denmark): *A Danish series on Hirschsprung s disease*

The male female ratio of the series of 53 patients with Hirschsprung s disease studied all cases diagnosed on a histological basis, was 3 1 in agreement with other similar studies. The incidence of associated anomalies was higher than reported in other studies and this might have a bearing on the high primary mortality The distribution of segment length was similar to those reported except in the extreme groups Girls were predominant in the ultrashort segment type no general distribution of long-segment patients as in C M Madsen s thesis for instance was seen. The incidence of anal atresia in girls was high in the present series

The results after definitive treatment show low mortality on Swenson s operation in agreement with previous findings

G H Willital (Erlangen-Nürnberg BRD): *Experiences with histochemical investigations of suction biopsies for Hirschsprung s disease*

About 200 children with aganglionosis are annually born in West Germany In order to receive proper treatment, such cases have to be diagnosed exactly and as early as possible Histochemical investigations of biopsies from the rectum are recommendable in view of a reliable diagnosis and of differentiation with regard to other types of megacolon The biop-

sies are feasible rapidly without anaesthesia on out patients and their reliability may be brought up to nearly 100% We have performed 67 suction biopsies on 61 patients revealing 19 cases of Hirschsprung s disease

The diagnosis is based on (*a*) absence of ganglia in Meissner s plexus (*b*) increased acetylcholinesterase in the muscularis mucosa and in the lamina propria of the mucosa (*c*) hypertrophy of extramural nerve fibres in submucosal tissue

At the biopsy some technical factors require special care (*a*) biopsy site above the anal canal corresponding to Campbell s investigations (*b*) adequate biopsy size (not less than 3 mm dia) taken by means of a special biopsy tube (*c*) no biopsies immediately after wash-out nor when the rectum is filled with hard feces Enterocolitis also introduces diagnostic difficulties

The biopsies may be evaluated in special departments they are transported in refrigerated boxes within 6 to 8 hours

J M Casasa & J Boix Ochoa (Barcelona Spain) *Treatment of total colic aganglionism*

A series of 37 cases of Hirschsprung s disease from a 6-year period at the Social Security Children s Clinic of Barcelona presented 6 patients with aganglionism of the entire colon and part of the terminal ileum An emergency operation was performed in all six owing to the intestinal occlusion picture they showed

Total colic aganglionism was suggested by absence of organic (obstructive or infectious) explanatory causes the sole finding being an ileal segment s sudden diameter change fairly adjacent to the ileocaecal valve exact diagnosis being derived from colon terminal ileum and widened ileal portion biopsies

The final operation was performed when the patient s general condition was good retrorectal transsphincteric pull-through of the normal ileum by Duhamel s technique without extirpation of colon as proposed by Rovíralta and by Duhamel Colectomy followed later when the child was entirely out of danger

This particular technique has the advantages of (*a*) a continent ileostomy (*b*) preservation of the colon to absorb water (*c*) a fast easy and non-traumatic procedure owing to preservation of the colon (*d*) exteriorization of the proximal colon through the operative wound enabling wash-outs to be made in the event of fecal retention (*e*) postponement of colectomy

This final operation was performed in 3 of our cases 2 patients survived and have already been colectomized now presenting a normal intestinal function at the age of 4 and 5 years respectively In the patient who was lost the aganglionism covered about 60 cm of the small bowel Another operation was necessitated by a postoperative complication (adherence at one of the biopsy sites) and the child did not recover therefrom

J Teixidor & M Andujar (Madrid Spain) *Sphincteromyotomy in ultrashort aganglionosis*

Megarectum secondary to anorectal spasm hypercontinence is treated by sphincteromyotomy More than 90 operations of this type were performed in the authors service since 1965 applying the technique of Bentley and Lynn with some modifications by Monereo such as interposition of mucous rectal membrane between both anorectal muscle margins avoiding recurrence by scar tissue formation

The diagnosis of short segment aganglionism is by no means easy In recent years histochemistry and suction biopsy have been helpful previously histological confirmation could only be obtained postoperatively This accounted for 76 cases In addition to pointing to these difficulties the aim of the paper was to study the clinico-radiological char

acteristics of short aganglionic cases and their follow-up (63 cases) over 6 months to 7 years showing a good final result in 61 cases and incontinence in two

Carlos Martinez Aimoyna R. & Eduardo Herrero Lopez (Madrid Spain) *Results with the delayed anterior hemianastomosis in Hirschsprung s disease*

Delayed anterior hemianastomosis is a modification of the coloanal anastomosis stage in the pull-through of Swenson s procedure for (1) avoiding leakage in the colo-anal anastomosis, (2) avoiding hypertonia of the internal sphincter and (3) avoiding a preceding colostomy The following stages are included. (1) Rectosigmoid or colic liberation (2) Vascular stage (we leave the first sigmoid portion in rectal aganglionism and the left colic in rectosigmoid aganglionism) (3) Inversion and pull-through of distal bowel (rectum) with a longitudinal posterior incision up to the skin margin (internal sphincterotomy) (4) Pulling the proximal bowel *through the distal bowel* (5) Colo-anal anastomosis in the anterior hemicircle (6) We leave this protrusion (under visual control) for 15 days then we resect it, (7) Posterior hemicircle anastomosis.

Out of 118 cases (from 1965–71) only 40 were treated by this technique The age distribution was 25% younger than 3 months 25% between 3 and 12 months and 50% older The most serious cases belonged to the youngest age group All patients lacked in weight and length The male female ratio was 3 1 The aganglionic segment was rectal only in 25% and rectosigmoid in 75% without any relation to clinical seriousness

A previous surgical procedure had been undertaken in 37 5% colostomy in 25% internal sphincterotomy in 5% ileocaecoplication and internal sphincterotomy in 5% and ileocaecoplication as sole treatment in 2.5% Nursing had been the sole treatment in 62.5% The total mortality was 5% (2 patients both younger than 3 months) due to sepsis.

The postoperative complications were intestinal obstruction (5 cases) intraperitoneal haemorrhage (2) peritonitis (1) perianal abscess (3) infection of laparotomy wound (3) evisceration (1) necrobiosis of the protrusion (6) and enterocolitis (6) two or three of these possibly occurring in 1 patient. Transverse colostomy became necessary in 6 cases of whom one (younger than 3 months) later died from sepsis

Follow-up after 1 year elicited colo-anal stenosis (10.5%) megacolon (5.2%) incontinence (5.2%) and no constipation in the 38 survivors Anal dilatation was performed in 3 cases, a fibrous ring dissection in one and sphincteroplasty in one case The latest review revealed a significant improvement in all these The other incontinent patient is waiting for definitive treatment.

J Boix-Ochoa (Barcelona Spain) *Anterior resection in Hirschsprung s disease (Rehbein s technique)*

Unlike State s method, Rehbein s technique involves partial resection of rectum and weakening of the anal sphincter It consists of (1) resection of the rectum performed approx 1–2 cm beneath the peritoneal floor with anastomosis 3–5 cm above the anus depending on age The anastomosis at such low level claimed to be unfeasible by some authors is a matter of surgical technique. (2) Weakening of the anal sphincter effected by energic dilatation pre or postoperatively in anaesthesia. (Several highly illustrative slides shown)

The author disagrees with the criticisms of Rehbein s technique as incomplete because a small aganglionic segment may be left. He has never encountered fecal retention secondary to occurrence of such unresected portions. He does not believe treatment of the anal sphincter s functional disturbance which is a constant concomitant of Hirschsprung s disease to be of paramount importance in view of good surgical results

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The diagnosis of short segment aganglion-ism is by no means easy In recent years histochemistry and suction biopsy have been helpful previously histological confirmation could only be obtained postoperatively This accounted for 76 cases In addition to point ing to these difficulties the aim of the paper was to study the clinico-radiological char

favourable prognosis in children with extra hepatic portal hypertension as compared with adult cirrhotics The good accessibility of oesophageal varices was also pointed out as well as the fact that their treatment is all that can be done before a certain age

Variceal sclerosis by Sotradecol was the main issue in the present series Submucosal injections of 3 ml at each session were given by oesophagoscopy once a month for the first 6 months and once every 6 months subsequently There were no complications. In the last 6 years 11 patients were treated, 10 of them with extrahepatic portal block.

One group of 5 patients had undergone previous shunts and had recurrent postoperative haemorrhages Thrombosed shunts (in 2 cases) were reoperated One patient had a Tanner procedure The other 4 patients stopped bleeding after injections

In the other group 6 patients no previous shunts had been used 5 of these were between 2 and 41/2 years of age Ligation of the coronary vein was combined with the first injection The pre-existing portal pressure averaged 37.3 cm H_2O an average 3 sessions of sclerosing injections sufficed to keep all patients non-haemorrhagic from 2 to 17 months after the second session

F Collado & F Castillo (Madrid, Spain) *Hepatorenal polycystosis with portal hypertension*

The various forms of polycystosis, according to Bernstein s classification and the relation of the disease with portal fibrosis were discussed The authors concur with the prevailing opinion that when both are present they are part of the same disease It represents a special characteristic if congenital hepatic fibrosis is found Portal hypertension only occurs in cases with portal fibrosis and is absent in those having cysts. An intrahepatic presinusoidal portal hypertension is concerned whereby the hepatic function remains normal This type of portal hypertension has a favourable prognosis The patients may live a normal life after a derivative operation which usually has been successful

Among 8 cases of hepatic renal polycystosis portal hypertension was only present in one boy aged 13 Biopsy made at diagnosis revealed a remarkable portal fibrosis, and splenoportography showed a typical pattern Four out of our 5 cases of infantile polycystosis were encountered in newborns the fifth concerned a child 4 years of age In all instances a slight portal fibrosis was elicited by hepatic biopsy

G A López Pérez & H E Beardmore (Montreal Canada Seville Spain) *Management of portal hypertension in children*

In about 50% of 44 cases of extrahepatic obstruction a careful anamnesis revealed a possible aetiological factor including omphalitis severe enterocolitis peritonitis and catheterization of umbilical vein during the neonatal period The average age of presentation was 4 years 9 months Haemorrhage was the initial symptom in 25 cases hepato- or splenomegaly was observed in 37 cases hypersplenism in 6, loss of weight in 4 and jaundice and ascites in 2 each Of 23 hepatic biopsies 16 revealed a normal liver the rest being diagnosed as minimal cirrhosis

Splenectomy was performed in 12 patients with recurrence of haemorrhages in all but one Of 18 cases subjected to splenectomy with splenorenal shunt 13 had a proper follow-up only one of these stopped bleeding. After Crile s procedure only 2 of 11 patients stopped bleeding Massive postoperative haemorrhages ensued on 3 portocaval shunts with one death Four of 5 patients who had a mesocaval shunt performed had no recurrent bleeding (6 years average follow-up) One jejunal interposition and one Able s procedure both failed to prevent further hae

In the author s series of 84 cases of the disease reviewed or operated on at the Children s Hospital of Bremen (Professor Rehbein) and at the Children s Hospital of Barcelona in 1963–72 excellent good and fair surgical results were recorded in 57 21 and 3 patients respectively

Panel II
Portal Hypertension

J Monereo (Madrid Spain) *Shunts for portal hypertension in children*

A total of 30 children younger than 11 years with massive bleeding from oesophageal varices were seen during the last 6 years A shunt from the portal region to the inferior caval system was made in 19 cases

As in most statistics reported extrahepatic block was more frequent than intrahepatic block however shunting was only possible in 14 out of 24 cases The rest were treated by several other techniques

The sole important complication of a shunt was recurrence of haemorrhage implying either thrombosis of the anastomosis or inadequate venous drainage The former may appear early to counteract it a continuous heparin drip through a small catheter left in the middle colic vein was used during 48 hours The catheter is useful for measuring portal pressures and for radiological control of patency of the anastomosis The latter complication is less important it may be suspected if the portal pressure fails to go down below half of the pre shunt level

Complete obstruction of the anastomosis may be suspected in the event of massive postoperative bleeding or ascites formation

In haemorrhagic recurrences and in patients not fit for a shunt operation only sclerosing injections of the varices are considered at the moment as the safest method of haemorrhage control until a better shunt can be applied Isolated splenectomy is strongly opposed at all events

Following these principles only 2 out of 30 patients were lost Both had been subjected to a major palliative surgical procedure on the oesophagus

W Clatworthy (Columbus Ohio) *Portal hypertension in children*

After 20 years 34 cases were submitted to this study Some kind of shunt was applied in 23 In the rest several other operations were tried but with worse results than those obtained with shunts In mesenterico-caval anastomosis a recurrence in 20% even several years after the operation suggests a more conservative prognosis 6 cases were reoperated.

Prior to placing a shunt treatment directed upon the varices is recommended

M Sulamaa (Helsinki Finland) *Portal hypertension*

Sixty five cases of portal hypertension in children were surgically treated and observed over a prolonged follow up period 67% of patients were younger than 7 years and the operation preceded this study by more than 11 years in 77%

74% of these cases are still alive the mortality occurred within the first 4 years and 67% of the deaths were in the extrahepatic group Final results according to different techniques Porto-caval anastomosis 64% survival Mesenterico-caval survival only 30% as in thoracic ligature of varices thoracic translocation of spleen gave good results in 25% in infants—the Hästbacka technique with partial resections of spleen is now preferred Infectious complications are common after these spleen translocations

J Diez Pardo (Madrid Spain) *Treatment of oesophageal varices in children*

The pathogenic features of oesophageal varices were discussed stressing their less un

ears to 5 years 6 months. The two epididymal tumours were cystic mesonephric remnants in boys aged 12 and 15 years

In all cases the tumour was first noted by parents or by family doctors at routine examination In only one case (of lymphosarcoma) was there an antecedent of undescended testis up to 5 months prior to the diagnosis

The treatment was simple resection of the two epididymal tumours and one of the cases of teratoma, and total orchiectomy in all the rest, combined with retroperitoneal lymph node cleansing in the rhabdomyosarcoma. In this case a single lymph node measuring 3 cm by 2 cm was considered invaded Actinomycin D and radiotherapy were administered

It is difficult to ascertain whether the lymphosarcoma was in truth primary since there was no local or regional involvement at the time of operation and the bone marrow was unaffected. The patient died 4 months postoperatively of generalized lymphoma.

The remainder of the patients are alive and free of disease including the rhabdomyosarcoma, in fact followed up for 5 months

F Aguilar (Madrid Spain) *Cavernous angioma of the mesentery*

A girl, aged 2 years 7 months complained of pain in the lower hemiabdomen provoked by palpation and presented an abdominal tumour with elastic consistency and extraordinarily mobile wherein three consecutive tumours could be discerned on palpation They were rounded about 5 cm in diameter Family history and radiological studies were noncontributory

A transverse infra-umbilical laparotomy was performed, revealing a series of mesenteric tumours necessitating resection of a length of ileum approximately 170 cm, the intestinal Magenstrasse" being restored by an end-to-end anastomosis.

According to the pathoanatomist s report the specimen was occupied by a series of globulous formations flowering out on both sides and occupying the reflection angle of the mesentery consistent with multicystic cavities in a honeycomb with dark-brown contents The intestinal lumen was found to be systematically permeable and unaffected. Higher up at the root of the mesentery there were some lymphatic gangia. The cystic formations were histologically judged to be lobes of a cavernous angioma, a dysembryoplastic formation in which basically the cavernous blood vessels participate An index of the malformative nature was the presence of masses of smooth musculature intermingled with vascular cavities yet not belonging to their walls The lymphatic ganglia submitted with the specimen were found to be undamaged and presenting merely lesions of an inflammatory nature

The girl is now 5 years old and her condition is normal

M G Guijarro (Madrid Spain)· *Cutaneous and intestinal haemangiomatosis*

The author reported a case of cutaneous and intestinal haemangiomatosis referred to him owing to gastrointestinal bleeding which started 6 years after surgical extirpation of a skin angioma. There were no other relevant signs in clinical and radiological examinations and laparotomy was carried out The operative finding was a true vascular tumour of the small bowel and which was resected

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It is believed that some children with oesophageal varices can be greatly helped with sclerosing injections particularly in the presence of recurrent haemorrhages after other surgical procedures have been tried

J Diez Pardo (Madrid Spain) *Aetiological factors in paediatric portal hypertension*

Discussion of the aetiological factors appearing in a series of 30 surgically treated patients (2–11 years old) with portal hypertension An intrahepatic block was present in 7 patients (23 3%) 6 of them true cirrhotics and the seventh suffering from congenital fibroadenomatosis Patients with prehepatic portal block were by far more numerous (23 cases 76 6%) including all cases with acquired lesions of the portal bed and those with congenital portal malformations Differentiation between the two last mentioned is virtually impossible

Particular attention was paid to omphalitis as a cause of extrahepatic portal lesions resulting later in portal hypertension Portal block is rarely encountered Experiments carried out in stillborn without hepatic pathology revealed in Monereo s experience a good communication between umbilical vein and vena cava via the duct of Arantius and which is superior to the communication of this system with the portal tree which may be filled with contrast material but invariably under a higher pressure The pressure gradients recorded in newborn also agree with these facts It is thought that omphalitis would cause portal thrombosis when the process is very severe and extensive which would be a fatal situation in all and any instances.

The thesis was illustrated by post-mortem studies on extended thrombosis from the umbilicus to the intrahepatic portal bed which resulted in the patient s death This is the sole exceedingly rare case in which omphalitis could be made responsible for thrombosis of the portal tree

E Moreno R Canales & M Hidalgo (Madrid Spain) *A new technique of mesenterico caval shunt for the treatment of portal hypertension*

In an attempt to avoid the problems associated with end to-side portocaval and side-to end mesentericocaval shunts we have experimented in dogs with a mesentericocaval shunt with interposition of an autologous internal jugular vein graft This technique was proved to be feasible in humans by performing it on ten cadavers

We have recently performed this shunt for the first time in an adult patient suffering from intrahepatic portal hypertension placing the vein graft between the duodenum and pancreas with a gratifying result

The same operation was once more successfully carried out on a boy of 12 years who suffered from portal hypertension and the Cruveilhier Baumgarten syndrome with abolishment of the haemorrhage In this case the graft was placed in an infraduodenal position

Free Papers

I Claret (Barcelona Spain) *Primary tumours of testis and epididymus*

Five testicular and two paratesticular tumours observed in the last 2 years were reported The testicular masses were two teratomas one rhabdomyosarcoma one primary testicular lymphosarcoma and one Sertoli s cell tumour The patients ages ranged from

years to 5 years 6 months The two epididymal tumours were cystic mesonephric remnants in boys aged 12 and 15 years.

In all cases the tumour was first noted by parents or by family doctors at routine examination. In only one case (of lymphosarcoma) was there an antecedent of undescended testis up to 5 months prior to the diagnosis.

The treatment was simple resection of the two epididymal tumours and one of the cases of teratoma, and total orchiectomy in all the rest, combined with retroperitoneal lymph node cleansing in the rhabdomyosarcoma. In this case a single lymph node measuring 3 cm by 2 cm was considered invaded Actinomycin D and radiotherapy were administered

It is difficult to ascertain whether the lymphosarcoma was in truth primary since there was no local or regional involvement at the time of operation and the bone marrow was unaffected. The patient died 4 months postoperatively of generalized lymphoma.

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L Martin-Sanz (Madrid Spain) *Paraduodenal hernia*

Internal hernia is considered highly infrequent although its true incidence is unknown owing to the fact that it is usually found either at laparotomy for intestinal obstruction of unknown origin or in post mortem sections

The author reported a paraduodenal internal hernia preoperatively diagnosed by radiological examinations with barium meal and by superior mesenteric selective arteriography The clinical signs and operative findings were described At the operation the hernical sac contained 3/4 of the entire small bowel The patient was cured by the operation and has been followed up for 10 months

C S Lopez Tello (Homburg/Saar BRD) *The significance of relaparotomy in biliary atresia*

Biliary atresia is found in one out of 16 000 births and the malformation is very rarely successfully treated by surgery Ladd (1928) observed at post-mortem in one of his incorrigible surgical cases lesions allowing an anastomosis By 1963 Hasse could collect 14 further cases and he reported two more of his own One more case has been reported subsequently

In the author s institution a girl was operated on who suffered from biliary atresia and the lesions found did not permit any type of anastomosis all extrahepatic biliary tracts being atretic The patient was re-explored some months later and a Roux-en-Y hepaticojejunostomy was performed

The operation cured the patient for the duration of 19 months whereupon considerable increase of bilirubin levels ensued and the patient s condition deteriorated progressively up to death in hepatorenal failure.

M Escudé-Casals H Fueyo-Gonzales & F Sanchez-Carranza (Barcelona Spain) *Testicle feminization syndrome*

There are two types the incomplete and complete type of the syndrome the former being characterized by a greater masculine differentiation of the lower genital tract with enlargement of the clitoris and the latter by a normal or small clitoris and normal appearance of the perineal structures A case of testicular feminization syndrome (of the complete type) in a girl aged 3 months with bilateral inguinal hernia was presented

The diagnosis was established by (1) histological study of the testes which showed immature testicular tissue (2) chromatin-negative buccal smear (3) chromosomal analysis, indicating XY karyotype and (4) vaginoscopy showing a short canal and absence of cervix The data gained concerning family history disclosed an excess of aunts on the maternal side

The surgical procedures are (*a*) not to remove the gonads during infancy (they are oestrogen producing and removal interferes with somatic and breast development) (*b*) extirpation of the testes as soon as the feminization process is completed in order to avoid the risk of degeneration (some authors disagree) (*c*) hormonal treatment with oestrogens after extirpation of gonads

The sex assignment should be female (owing to impossibility of forming a phallus and inducing normal breast development) Psychological orientation is necessary The patient may marry but remains sterile The parents should be made aware that the ratio of normal females normal males and affected males is 2 1 1

G Utrilla, F Negro & F Berchi (Madrid Spain) *Hepatic echinococcus cysts in children*

Out of 74 cases of hydatid disease in children surgically treated at the Department of surgery Clinica Infantil La Paz, 1965–72, 31 cases were hepatic

Plain X-rays of the abdomen allow diagnosis in calcified forms only (6 cases in the present series) Therefore contrast studies (liver scan, angiography cholecystogram) were diagnostically more useful. Immunological tests were of limited interest.

Marsupialization is no longer accepted as management, total extirpation of the cyst with adjacent layer being the treatment of choice

The operative findings in the present series were: solitary cyst with thick pericystic in 61% closed infected cyst in 3 2% cyst perforating into the peritoneal cavity in 3.2% and multiple cysts in 29% The surgical techniques used were: emptying of the cyst and closure of the cavity in 5 cases emptying of cyst with partial pericystic resection in 12 cases "in toto" resection of cyst and surrounding layer in 10 cases and hepatic resection or lobectomy in 9 cases

The authors accept surgery as the only means of achieving total cure of the disease with a very low complication rate and with reduced hospitalization time

L. Morales Fochs (Barcelona, Spain): *Multilocular renal cyst*

At a routine examination a palpable mass was found in the left hemiabdomen of a 1 year-old boy X ray studies revealed presence of an intrarenal tumour with major calyceal distortion suggesting diagnosis of Wilms s tumour Nephrectomy was performed.

The reason for presenting this case was the difficulty of differential diagnosis with regard to Wilms s tumour particularly when no pre operative chemotherapy or radiotherapy has been applied. The opinion is not uncommon based on the histological similarity of this lesion and Wilms s tumour that the cysts in question represent a cured or abortive form of the tumour

B E Mugica & F Gutiérrez (Madrid Spain): *Superior femoral epiphysiolysis in new born*

The authors reported 4 infants with femoral superior epiphysiolysis representing 6% of all obstetric lesions of this type which are more frequently observed in superior humeral and inferior femoral location (81% and 9% respectively) All patients were podalic presentations as in the majority of published series

Differential diagnosis is necessary with regard to congenital luxation of the hip congenital coxa vara and arthritis of the hip The treatment is based upon. orthopaedic reduction and plaster cast in maximal abduction 90 degree flexion and 15 degree internal rotation The authors did not use continuous traction

J Cuadros L. Quiros & P Jover (Madrid Spain) *Congenital malformations of the tongue*

Congenital malformations of the tongue were studied regarding relationship with other system malformations occurring in the same period of intra-uterine life (limbs brain lips etc) The following cases were reported.

(*a*) One case of the Papillon-Leage-Psaume syndrome with typical malformations of tongue face and fingers among the chromosomopathies

(*b*) In the embryopathic group one case of glossodynia with severe respiratory distress with post-mortem study

(*c*) Five further cases constituting the group of 4th to 6th week embryopathies. glossopalatal ankyloglossia, inferior ankyloglossia, aglossia-adactylia Hanhart s syndrome all defined by oral and extremity-associated

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malformations One of the two cases of Hanhart s syndrome presented associated anorchia and anal atresia

(*d*) 53 cases of the Pierre Robin syndrome classified into the malposition group and

(*e*) 14 cases of macroglossia 8 angiomas 2 lymphangiomas and one tumour of Abrikossoff all in the abnormal growth group

S Ruiz Company A Vilariño & V Sancho (Valencia Spain) *Bilateral renal thrombosis*

Thrombosis of the renal vein in infancy is a serious impairment requiring early diagnosis and treatment Its prognosis remains fatal in most bilateral cases The authors are aware of only three survivals reported after bilateral thrombosis and a fourth case was therefore presented

A male infant of 15 days old had on admission gastroenterocolitis severe dehydration uraemia oligoanuria and enlargement of both kidneys An angiographic study disclosed thrombosis of renal vein and inferior vena cava On thrombectomy the thrombi in the vena cava and in both renal veins could be removed Diuresis started after 10 hours the postoperative course was uneventful and the renal function was restored The infant was in a good general condition 4 months later

The importance of early surgical treatment was emphasized in view of the high mortaility rate in conservative treatment of bilateral renal thrombosis in the neonatal period

J Alba Losada J A Velazquez D Moneva & C Cirajas (Valencia, Spain) *Technetium-99 examination of Meckel s diverticulum*

The use of the Tc 99 isotope was recently introduced with satisfactory results in the authors Clinic as a complement to the conventional procedures in cases of rectal bleeding There were 5 cases of proven Meckel s diverticulum in the first year of our surgical service Tc 99 was used in two of them with encouraging results

The first case was that of a male child with a long standing history of rectal bleeding and negative complementary data Tc 99 examination revealed a clear and definite diverticulogram the diagnosis was confirmed after laparotomy

In the second case a male child was admitted to the emergency room because of abdominal pain and rectal bleeding A radiodiagnostic study suggested a large Meckel's diverticulum On radioisotope study a broad diffuse captation compatible with a large diverticulum was elicited This diagnosis was also confirmed in the operating theatre

In the other three cases the use of Tc 99 was not possible Two of them presented an acute pattern of intestinal obstruction the third who had a serious haemorrhage required immediate surgery

C A Banuelos C Estellens & M Sanz (Madrid Spain) *Echinococcosis in children*

The authors reported the experience in hydatid disease of the Service of Paediatric Surgery of the Provincial Hospital F Franco Madrid covering the period Oct 1st 1969 to Dec 31st 1971 during which 16 patients with a total of 26 cysts were treated Ten of these patients suffered from solitary lung cysts 2 from double lung cysts 2 from multiple hepatic cysts and only one from cysts in liver and lung both The pulmonary localisations were three times as frequent as hepatic ones Patients younger than 6 years constituted 8% of the total series (ranging from 21/2 to 12 years) 11 patients were boys and only 5 were girls

O Knutrud (Oslo Norway) *Parenteral alimentation*

In the last 2 years 60 newborn and infants were treated by total parenteral hyperali-

nentation for periods averaging 9 days The mortality rate was 16% The indications were variable from complicated malformations to postoperative complications A specially designed intravenous diet was studied and adapted to commercially available preparations.

A criticism of these commercial preparations was presented emphasizing Intralipid 20% Vitrum and Aminofusin and the new Vamin Vitrum having an E/T ratio similar to that of breast milk

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC ENDOCRINOLOGY

12th Annual Meeting Bergen June 21–24 1973

A M Bongiovanni T Moshang Jr J Parks & V Vaidya (Philadelphia Pa) *Adrenogenital syndrome later consequences*

Information on the later years of individuals with congenital adrenal hyperplasia is limited Among untreated cases in the fourth decade of life and beyond there are often vague complaints such as abdominal pain weakness impotence in males and on occasion periodic fever and diabetes

Surprisingly females first treated as late as in the third and fourth decade of life rapidly feminize menstruate regularly and in a few limited studies reveal normal cyclic hormonal patterns There is increasing evidence that permanent physiologic impairment of the female hormonal pattern after exposure to androgens in early life as noted in rodents does not apply to higher anthropoids and man Many pregnancies are now reported but the precise incidence of fertility in treated females is unknown However several features of these pregnancies are noteworthy a high incidence of spontaneous abortion tubal pregnancy and Caesarean section (usually because of vaginal scarring from earlier surgery) live infants who are delivered have been normal in all respects there is a high ratio of female/male infants produced by women with this syndrome (about 2 4 1 0) lactation has been normal Information on the fertility of adult males is meagre and no generalizations are possible There is a certain incidence of testicular tumours of probable adrenocortical origin in untreated males On occasion adenomas of the adrenal have been described in untreated cases and very rarely carcinomas

John Money (Baltimore Maryland) *Behavioral outcome after early cortisone therapy in the adrenogenital syndrome of 46 XX hermaphroditism*

Genetic females with the adrenogenital syndrome who were genetic females neonatally diagnosed and appropriately treated hormonally surgically and socially as females differentiate a postnatal feminine gender identity If treated hormonally surgically and socially as males they differentiate a postnatal masculine gender identity The femininity of those who live as girls is characteristically tomboyish In childhood they enjoy competitive energy expenditure with boys in sport but they are not belligerently aggressive They lack juvenile interest in spontaneous doll-play rehearsals of motherhood They are high academic and vocational achievers and early set their sights on a non-domestic career in preference to or in parallel with marriage and a domestic career Cortisone therapy notwithstanding their menstrual onset is one or more years later than average They lag behind their age mates in reaching the developmental stage of romance dating and boyfriends despite their usually outstanding good looks They have a very low incidence of re-

ported adolescent masturbation and of bisexual imagery or activity They do not report adolescent orgasm dreams. Pregnancy and lactation are possible as is the establishment of an adequate mother-role despite initial dif fidence In adulthood those patients reared as boys virilize under appropriate endocrine treat ment, and are able to establish a masculine sex life even if handicapped by a too-small phallus. Irrespective of sex of rearing, symptoms of behavioral disability are infrequent and are related more to childhood home life than to the adrenogenital condition *per se*

Maria L New Sigrun Korth-Schutz & Lenore S Levine (New York): *Post-pubertal virilism in girls with treated congenital adrenal hyper plasia*

Although most females with congenital adrenal hyperplasia (CAH) treated from early life with glucocorticoids undergo a normal puberty and may later reproduce some girls manifest viril ism after the onset of puberty Two sisters who had CAH due to 21-hydroxylase deficiency were studied extensively One treated from age three with glucocorticoids was studied at age twenty-three because of secondary amenor rhea, deepening voice and conspicuous facial hair The other sister treated from birth was studied at age thirteen because of primary amenorrhea, conspicuous acne and facial hair Both sisters excreted large amounts of 17 ketosteroids on doses of glucocorticoids which had previously kept urinary ketosteroids at a fairly normal level Endocrine studies revealed that urinary 17-ketosteroids did not suppress below 10–12 mg/day even after dexamethasone (8 mg) was administered daily for 6 days High base line urinary estrone levels and plasma testosterone levels were suppressed to normal female levels by this dose but not to levels expected in normal females treated with large doses of dexamethasone While adrenals were suppressed with continued dexametha sone treatment neither Norlutin nor HCG administration resulted in a change of the uri nary 17 ketosteroid or plasma testosterone levels Laparoscopy revealed bilateral sclerotic ovaries in both sisters These endocrine studies did not prove that the unsuppressible androgen was ovarian in origin Although the ovaries were anatomically abnormal physiological abnormality was not demonstrated Thus the cause of the post-pubertal virilization and sclerotic ovaries in two sisters with treated CAH remains to be elucidated. Although sclerotic ovaries are often said to be associated with CAH endocrine data are sparse Could the ovarian pathology at puberty observed be an effect of excessive adrenal androgen in utero?

C G D Brook A. Prader M Zachmann & G Mürset (Zürich) *Treatment of congenital adrenal hyperplasia*

Submitted for publication to *J Pediat*

M T Pham-Huu-Trung M Gourmelen & F Girard (Paris). *The simultaneous assay of cortisol and 17α-hydroxyprogesterone in the plasma of patients with congenital adrenal hyperplasia*

K Rager U Schmoldt, D Gupta & J Bierich (Tübingen) *Plasma levels of 17-OH-proges terone substance S and deoxycorticosterone in children with CAH measured by simultaneous radio-immuno-assay*

The relative importance of the circulating 17 OH-progesterone (17-OHP) Reichstein s substance S (S) and 11-deoxycorticosterone (DOC) which are the precursors of cortisol and corticosterone respectively in the plasmas of child patients with congenital adrenal hyperplasia (CAH) remains unclear In an attempt to study this methods have been developed for the simultaneous measurement of these three

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reached at 120 min while peak values of full-term infants were reached at 75 min following ACTH injection. Before ACTH E values were higher in premature than in full-term infants. However there was no significant rise after ACTH. In contrast E of full-term infants peaked 60 min post adrenal stimulation Some babies with low gestational age (less than 30 wks) showed low F (3 13 μg%) E (5.59 μg%) and B (3.82 μg%) values and no rise of plasma steroids at all

These data suggest a lack of adrenal response to ACTH in early gestation a delayed and sluggish response in late gestation and a normal response at term compared with data in adults.

S. Leisti & J Perheentupa (Helsinki): *Two-hour ACTH-test in the diagnosis of adrenocortical failure in children*

Plasma cortisol response to a single i v injection of tetracosactide 0.25 mg/1 73 m² was studied in 102 children with normal adrenocortical function 22 with defective secretion of ACTH 21 with the syndrome of autoimmune endocrinopathy and moniliasis with or without clinical Addison s disease and 24 with recent pharmacological glucocorticoid therapy The test was repeated in 84 children to determine its precision.

The means and 95% confidence intervals of plasma cortisol levels had to be calculated after log transformation, whereas the increments showed normal distribution In the reference children, the mean basal (8 a m.) 60 120 and 180 min plasma cortisol level was 22 46 50 and 39 μg/100 ml (95% confidence interval 11–47 33–63 35–70 and 24–63 μg/100 ml) respectively The mean 60 120 and 180 min increment was 23 26 and 16 μg/100 ml (7 to 39 8 to 45 and −3 to 35 μg/100 ml) respectively The normal response was defined by these three parameters.

The precision calculated as the SD of the relative intra-pair differences of individual test pairs was 17.5 8.1 and 22.7% for the basal level two hour level and two hour increment respectively

Seven of the 22 children with defective ACTH secretion had normal response by all 3 criteria. Irrespective of the severity of the secondary adrenocortisal failure all had normal 2 hour increment

Eight of the 21 patients with endocrinopathy syndrome had normal response by all 3 criteria, and 6 were defective by all 3 criteria An intermediate group of 5 had normal basal level but no increment. Evidently they were under maximal endogenous ACTH-stimulation During follow-up this intermediate group was seen to progress to deficiency by all 3 criteria.

The children with recent pharmacological glucocorticoid therapy were similar to patients with defective ACTH-secretion except for 3 who developed failure by all the 3 criteria During the recovery the combination of normal basal level and no increment was never seen

The 2-hour ACTH-test proved to be an accurate and relatively precise test for the diagnosis and follow-up of a failure of the pituitary adrenocortical axis

A Kowarski L. de Lacerda & C J Migeon (Baltimore Maryland): *Effect of ACTH on the diurnal fluctuations of plasma aldosterone*

A portable non-thrombogenic constant blood withdrawal pump was used for a continuous blood sampling on 6 normal male subjects The men were allowed to continue normal activity for the duration of the experiment Blood was collected every 30 minutes for a 24 hour period The activity and posture of each subject during each collection period was recorded.

The integrated concentrations of cortisol, and aldosterone fluctuated widely a significant correlation was found in 2 subjects who spent more than 50% of the test period in bed ($p<$ 0 025 and $p<$0 0005) In 3 other subjects who had spent much less of the test period in bed

steroids from a single plasma by radio-immuno-assay technique The alkaline plasma was first extracted and this extract after neutralization purified through a 2 step silica gel TLC This was followed by acetylation of the sample and a further TLC which produced individual fractions without any measurable contaminations For radio-immuno-assay a single antibody was used which had the following cross reactions 17-OHP 100% S 100% and DOC 39% In most normal subjects these three steroids were present in plasma but in low concentrations In all the 18 subjects with CAH due to 21 hydroxylase deficiency the plasma concentration for 17-OHP was markedly elevated The levels of S and DOC were low in the patients and in several cases could not be detected With ACTH administration the concentration of 17-OHP reached levels 3 to 4 times those of the control samples whereas S and DOC concentrations had only slightly increased When related to cortisol these values produced a reliable diagnostic tool for the quick detection of the type of hydroxylase defect in children suffering from CAH

K von Schnakenburg (Kiel) F Bidlingmaier & D Knorr (München) *Quick diagnosis of congenital adrenal hyperplasia (CAH) due to 21 hydroxylase by radioimmunoassay for 17 alpha hydroxyprogesterone*

For 17 Hydroxyprogesterone (17-OHP) two assays using a radioimmunological technique were developed The antiserum (diluted 1 10000) to 17-OHP 3-carboxymethyloxime bovine serum albumin revealed a significant cross reaction with progesterone 19 other steroids with similar chemical structure or at high plasma levels showed negligible crossreactions

The first and highly specific method includes extraction of plasma with ether column chromatography on Sephadex LH 20 radioimmunoassay (RIA) and correction of procedural losses by a tritiated 17-OHP internal standard The second simplified method consists of extraction of plasma samples of 0 1 and 0 25 ml with methylene chloride followed by RIA

The normal range of 17-OHP levels of about 100 ng/100 ml can be measured correctly by the specific method only However the diagnosis of CAH as well as the control of therapy can be done quite well by the simplified method of which the results are obtainable within approx 6 hours In treated CAH plasma levels of 17-OHP of between normal and about 10000 ng/100 ml are found depending on therapy In untreated CAH the 17-OHP levels are usually above 10000 ng/100 ml When plasma levels of 17-OHP in CAH were assayed by the 2 radioimmunoassays and by a gas-chromatographic method a good couelation was found

R P Willig & W Blunck (Hamburg) *Cortisol cortisone and corticosterone in the plasma of premature new born infants following ACTH stimulation*

It is uncertain whether the adrenal gland of premature infants responds to ACTH adequately To our knowledge nothing is known about the velocity and the maximal rise of plasma steroids after ACTH stimulation in prematures immediately after delivery To investigate these questions an attempt was made to compare serial plasma levels of cortisol (F) cortisone (E) and corticosterone (B) of 15 premature infants (28th–36th wk 490–2350 g) with those of 15 full term infants Postpartal blood samples were obtained usually by heel-stick before and every 15 min after i m ACTH injection within the first 6 hours In every baby 8–10 determinations were performed After paper chromatography F was measured by CPB technique E and B by radio-immuno-assay

So far 15 premature neonates have been studied Mean F values before ACTH stimulation were higher B values lower than in full-term neonates There was a delayed rise of F and B in premature infants with peak values

continuous decrease in DHT/T ratio A significant rise in E_2 is seen in girls from age 11 while a lesser but evident rise is also observed in boys by 14 to 15 years of age (P_3–P_4)

The early increase of plasma free DHA followed by a significant rise in Δ and E_1 around 8 to 9 years of age long before any increase in T DHT or E_2 suggests a possible role of these hormones in the activation of the hypothalamo-pituitary gonadal axis. The rise observed for these hormones at prepuberty earlier in girls than in boys consistent with the earlier onset of puberty in females could suggest that these hormones may play a role at least permissive in the regulation of the gonadostatic threshold to feed-back effect of circulating sex hormones

J M Tanner R. H Whitehouse W A Marshall & B S Carter (London)· *Equations for the prediction of adult height from height and skeletal maturity at ages 3 to 16*

To be submitted to *J Pediat*

R. Steendijk (Amsterdam) *Excessive height in girls some contributing factors*

E. A Werder G Mürset M Zachmann C G D Brook & A Prader (Zürich)· *Treatment of precocious puberty with cyproterone acetate*

Submitted for publication to *Pediat Research*

E. E Joss & K A Zuppinger (Berne)· *Diagnostic value of anthropometric parameters in growth retarded children with special reference to partial GH-deficiency*

Height height velocity bone age (Tanner) diaphyseal diameter and the cortical thickness of the metacarpal bones were analysed in 26 patients with GH-deficiency (peak plasma GH during ITT <2 ng/ml) 27 patients with constitutional growth retardation (peak plasma GH >7 ng/ml) and 7 patients with low birth weight dwarfism (LBW)

For height the best demarcation line between GH-deficiency and const growth retardation was −3.5 S D with six overlaps especially in younger patients. In LBW height had a wide scatter from −2.2 to −6 0 S D

Height velocity divided patients with GH deficiency from const growth retardation and LBW at all ages with only five exceptions at an S D of −2.5

For bone age the best demarcation line was at an S D of −3.5 but there was a considerable overlap especially in young patients

Diaphyseal diameter of the metacarpal bones is a poor differentiating parameter cortical thickness however divided patients with GH deficiency from const growth retardation quite efficiently at an S D of −2 2 with only two exceptions

A group of 6 patients had intermediate peak plasma GH values during ITT (2.3 to 4.2 ng/ml) On repetition of the ITT and on an i v glucose and arginine test the peak plasma GH values were again in an intermediate range These 6 patients therefore were considered having partial GH-deficiency All anthropometric data of these patients were in an intermediate range between patients with 'total GH-deficiency and const. growth retardation which is in agreement with the assumption of partial GH-deficiency

O Butenandt A Keich & E Rajmann (München and Garmisch-Partenkirchen)· *Rheumatoid arthritis growth and growth hormone*

The growth rate of children with severe rheumatoid arthritis, especially when the disease begins in the first years of life may be highly depressed This is partly due to a therapy with corticosteroids but also secondary to the chronic disease itself

In 16 children with rheumatoid arthritis and dwarfism growth hormone in plasma was meas-

the correlation between the integrated concentrations of aldosterone and cortisol was significant only in one ($p<0.05$)

The effect of an intravenous infusion of synthetic ACTH on plasma aldosterone was investigated in 16 normal subjects The levels rose from (mean±S D) a baseline of 5±3 to 18±ng/100 ml 30 minutes after the ACTH infusion The concentration of cortisol rose at the same time from 7 7±2 6 to 23 4±3 0 micrograms/100 ml±S D

It was concluded that physiologically ACTH along with other factors plays a significant role in the regulation of the level of aldosterone in man This effect becomes more evident during periods of supine posture when the effect of body position on the levels of aldosterone is minimal

C C Forsyth D C L Savage & J Cameron (Dundee) *Adrenocortical function in normal precocious and delayed puberty*

There is a steady rise in the 24 hour urinary excretion of the 17 hydroxycorticosteroids and more specifically of the α ketolic metabolites of cortisol thoughout infancy childhood and adolescence which is directly related to body weight

In contrast there is a steep rise in the 24 hour urinary excretion of the 17-oxosteroids and more specifically of dehydroepiandrosterone, aethiocholanolone and androsterone in relation to puberty This increase is much greater than could be explained on a body weight basis and it occurs late in delayed puberty and early in precocious puberty

The metabolic degradation which takes place chiefly in the liver of the adrenal androgens alters at puberty favouring the production of 5α-steroids rather than 5β-steroids The rise in the 5α/5β ratio with age for the 17-oxosteroids measured by our method is statistically significant and the rise in this 5α/5β ratio occurs early in precocious puberty

Data were presented from 62 normal infants children and adolescents and in addition from 10 patients with early or precocious puberty and from 7 patients with delayed puberty on which the above conclusions are based

The changes in adrenocortical function at puberty are thought to be associated with activity of the gonadal axis but the precise relationship is not fully understood

J R Ducharme G Alberti M G Forest E de Peretti M Sempé & J Bertrand (Lyon) *Pattern of plasma androgens and estrogens from childhood through adolescence*

Little information is available on the pattern of androgens and particularly estrogens throughout childhood and adolescence We have measured by specific radioimmunoassays plasma androstenedione (Δ) testosterone (T) dihydrotestosterone (DHT) dehydroepiandrosterone (DHA) estrone (E_1) and estradiol (E_2) in 125 normal children 4 to 17 years of age Each subject was classified P_1 to P_5 according to genital development described by Tanner and bone age estimated

Plasma free DHA increases as early as 6 years of age in girls and possibly slightly later in boys followed by a gradual rise throughout childhood and adolescence Δ increases significantly from age 9 in girls and one year later in boys with a steady preponderant rise thereafter in girls A similar pattern is found for free E_1 with an early increase by 8 to 9 years of age. In girls a more significant rise from age 11 and a peak increase around 12 to 14 years (P_3–P_4) are observed In boys a significant increase at approximately 13 to 14 years of age is found. T and DHT levels are similar in both sexes until 11 years of age when a rapid increase occurs in girls who reach adult values by age 12 to 14 In contrast there is no significant increase in plasma T and DHT levels in boys until 14 years of age (P_4) but these values continue to rise throughout adolescence with a

ured before and following insulin induced hypoglycemia 3 children not treated with corticosteroids prior to the test had basal growth hormone levels of 0 5 12 4 and 41 ng/ml plasma Following hypoglycemia they reached a maximal growth hormone level between 18 6 and 28 3 ng/ml The other 13 patients receiving corticosteroids for long time had fasting growth hormone levels of 0 7±0 7 ng/ml and a maximal rise to 8 4±6 7 ng/ml Six patients responded with a normal rise to hypoglycemia 7 patients had a poor response (less than 5 ng/ml) Twenty healthy children with normal growth rate comparable in age sex and stage of puberty had fasting growth hormone levels of 2 6±2 0 ng/ml and a maximal rise to 17 5± 7 9 ng/ml following hypoglycemia

The growth hormone response to hypoglycemia in the corticoid treated group of patients differs significantly from the few untreated patients and the healthy children However there was no correlation between severity of growth retardation growth hormone levels and its response to the hypoglycemic stimulation

Dacou Voutetakis Th Carpathios N Logothetis & N Matsaniotis (Athens) *Evidence of defective growth hormone secretion in microcephaly*

Spontaneous nychthemeral and provoked growth hormone (GH) secretion was examined in 5 children with familial or idiopathic microcephaly GH was determined by radioimmunoassay in samples obtained hourly during a 24-hour period and following oral glucose load and/or insulin induced hypoglycemia In 3 of the 5 microcephalics GH levels did not exceed 3 ng/ml either during sleep or significant hypoglycemia In one of them no secondary sex characteristics were present at the age of 15 years Two of the non responders were brothers The thyroid function was normal in all cases It is suggested that familial or idiopathic microcephaly might represent an example of developmental arrest of the brain associated with pituitary hypofunction Supportive evidence is derived from analogous neuro-endocrine complexes thus far reported septo-optic dysplasia with defective growth hormone secretion (de Morsier s syndrome) and absence of the olfactory bulbs associated with deficiency of gonadotropins and probably of MSH (de Morsier Kallman s syndrome)

J Perheentupa S Autio S Leisti & C Raitta (Helsinki) *Cerebral malformation with tapeto-retinal dystrophy and growth hormone deficiency in two brothers a new syndrome*

M G Forest A M Cathiard P C Sizonenko & J Bertrand (Lyon and Geneve) *Hypophyso-gonadal function in infants during the first year of life*

Testosterone (T) LH and FSH have been *measured by specific radioimmunoassays In cord blood* there is a sex difference in plasma T levels the values being (mean±1 S D) 33 2± 9 8 and 25 7±8 2 ng/100 ml in 29 males and 36 females respectively *In male infants* we already reported an increase *in mean T* levels from birth to the second week of life A further increase in T concentration was observed in 33 normal male infants with a peak between the second and third month of life followed by a gradual decrease (1–3 months=198 2±65 7 ng/100 ml 3–5 months=87 5±56 6 ng/100 ml 5–7 months=21 47±20 6 ng/100 ml) After the 7th month of life values observed (7.58± 5 9 ng/100 ml) reached those of prepubertal boys (6 62±2 46 ng/100 ml) *In 27 female infants* the pattern was very different T concentrations dropped rapidly from birth to the third week of life reaching values (7 09±3 9 ng/100 ml) similar to those of prepubertal girls (6 58±2 48 ng/100 ml)

FSH was significantly higher in female (9 7± 2 8 mU/ml) than in male infants (7 6±2 66) In contrast LH was higher in males (23 2±

Sm. in maternal serum, cord blood and amniotic fluid has been determined with the method previously described

Human chorionic somatotropin (HCS) was also measured in the samples of maternal serum and cord blood by radioimmunoassay

In our initial studies of 10 mothers and their newborn infants the findings can be summarized as follows.

Sm. in maternal serum in late pregnancy is within normal reference limits but with great individual variations. However corresponding values in maternal serum and cord blood were equal

Sm. was present in amniotic fluid in amounts found in maternal serum.

Although the infant with the highest birthweight had the highest value of Sm apparently no correlation was found between Sm and birthweight

A certain relationship seems to exist between Sm and HCS in maternal serum in the sense that the lowest values of Sm was found in the mothers with low concentration of HCS in plasma

K W Kastrup (Copenhagen)· *The relationship between Somatomedin glucose and insulin studied in vivo and in vitro*

Previously we have reported our studies on the relationship between somatomedin (Sm) HGH and insulin and demonstrated changes in the amount of Sm. connected with changes in plasmainsulin The observations were not conclusive and in attempt to clarify these the following studies were done

Insulin tolerance test were performed in 15 patients with various types of growth retardation. Glucose Insulin IRGH and somatomedin was measured The initial phase was studied particularly since we reported earlier on our findings in the later phase An immediate fall in Sm activity was found in all patients In patients with growth retardation of nonhypopituitary origin this was followed after 20 min by a rapid increase in Sm. back to initial values.

In hypopituitary patients the fall in Sm continued for the following period and did not return to initial values

Identical variations was found in plasma glucose

The findings reflects true changes of Sm *in vivo* or altered conditions for the assay in vitro

P Saenger E Wiedemann S Korth-Schutz J E. Lewy R R Riggio A L. Rubin K H Stenzel E Schwartz & M I New (New York Bronx)· *Serum somatomedin (SM) and growth in chronic renal failure and after renal transplantation*

As growth is markedly retarded in chronically azotemic children despite normal growth hormone levels serum SM activity in patients with chronic renal failure was examined. Hormonal and metabolic factors influencing growth were studied in 9 uremic children who received renal homografts While virtual growth arrest was present prior to transplantation in all except one post transplant growth velocity based on bone age (GVBA) became normal in four (88–103%) accelerated in two (127–139%) and remained subnormal in three (18–50%) Serum somatomedin (SM) now recognized as an important factor in linear growth was very low in all children before transplant (0.39±0 12 U/ml) with levels in the hypopituitary range but rose in each child after transplantation (0 83±0 16 U/ml) reaching the normal range in all except two Post-transplant GVBA was not related to glucose tolerance growth hormone response to arginine-insulin stimulation thyroid function and plasma FSH and LH levels but showed significant positive correlations ($p <$ 0 05) with both serum SM activity and creatinine clearance No significant correlation

These facts indicate that the regulation of pituitary secretion is probably different for TSH and LH FSH and suggest that the presence of a normal pituitary reserve of gonadotropins may be correlated to a normal exposure to endogenous hypothalamic luteinizing hormone releasing hormone On the other hand the absence of a pituitary reserve of gonadotropins before the age of puberty may allow a precocious diagnosis of gonadotropic deficiency

P C Sizonenko L Paunier & A Cuendet (Geneva) *Evaluation of the hypothalamic pituitary gonadal axis by a new anti androgen (SCH 13521) in boys*

In order to evaluate the gonadotropins (FSH and LH) and testosterone (T) response to a new anti androgenic substance (SCH 13521) 18 boys were given 5 mg/kg of SCH 13521 for 5 days (day 1 to 5) Plasma FSH LH and T were determined before during (day 5) and after the administration of SCH 13521 (day 6)

In 4 prepubertal boys mean basal FSH LH and T levels were 3 5±0 3 mU/ml (MRC 68/39) 6 7±1 7 mU/ml (MRC 68/40 and 95±6 5 ng/100 ml No significant change in FSH LH and T was observed with SCH 13521 In 14 pubertal boys (stage P2 to P5) mean FSH (4 2±0 6 mU/ml) increased to 5 2±0 8 ($p<$ 02) and 5 8±0 8 ($p<$ 005) on day 5 and 6 respectively LH (8 2±0 9 mU/ml) to 10 2±1 1 ($p<$ 05) and 11 4±1 3 ($p<$ 001) T (412±71 ng/100 ml) to 624±144 ($p<$ 05) and 661±102 ($p<$0 001)

It may be concluded that this new anti androgen increases gonadotropins and testosterone levels in pubertal boys therefore allowing the assessment of the hypothalamic pituitary gonadal axis when puberty is present

J C Job P E Garnier J L Chaussain J E Toublanc & P Canlorbe (Paris) *Pituitary gonadotropic response to luteinizing hormone releasing hormone (LH RH) and testicular response to chorionic gonadotropin (HCG) in children with undescended testes*

Thirty nine prepubertal boys with undescended testes and without other major anomalies (16 unilateral 23 bilateral cases) have been submitted 1) to evaluation of pituitary gonadotropins (FSH and LH) in the serum 0 to 90 min after venous injection of synthetic LH RH 0 1 mg/m² and 2) to evaluation of plasma testosterone before and after stimulation with 3 injections of HCG 1500 U every other day With both tests no significant difference was observed between unilateral and bilateral cases

Mean basal levels of FSH and LH and mean peak level of FSH after LH RH were not significantly different from the means of normal prepubertal controls The mean peak level of LH after LH RH was significantly ($p\leq$0 01) lower than that of controls Two patients did not respond at all to LH RH and could be considered as hypogonadotropic

Mean and individual plasma basal levels of testosterone in ectopic boys were similar to those of controls But mean testosterone level after HCG was significantly ($p<$0 01) lower in the patients (220 ng/100 ml±S E M 25) than in prepubertal controls (346 ng/100 ml±S E M 49) and 14 out of the 39 patients had a peak testosterone level under the normal prepubertal range As mean post stimulatory levels of testosterone were found similar in ectopic patients and in controls after the onset of puberty the difference observed before puberty could be correlated to a delayed endocrine maturation of ectopic testes

These data lead to discuss the possible relations between gonadotropin pituitary reserve and testicular response to HCG in prepubertal boys with ectopic testes

H J Andersen K W Kastrup & P E Lebech (Copenhagen) *The possible role of Somatomedin in the growth of the human fetus*

Observations on the occurrence of somatomedin (Sm) in the human fetus have to our knowledge not been reported previously

Sm. in maternal serum cord blood and mniotic fluid has been determined with the method previously described

Human chorionic somatotropin (HCS) was lso measured in the samples of maternal erum and cord blood by radioimmunoassay

In our initial studies of 10 mothers and their newborn infants the findings can be summarized as follows.

Sm in maternal serum in late pregnancy is within normal reference limits but with great individual variations However corresponding values in maternal serum and cord blood were equal

Sm was present in amniotic fluid in amounts found in maternal serum.

Although the infant with the highest birthweight had the highest value of Sm. apparently no correlation was found between Sm and birthweight.

A certain relationship seems to exist between Sm. and HCS in maternal serum in the sense that the lowest values of Sm. was found in the mothers with low concentration of HCS in plasma.

K. W Kastrup (Copenhagen): *The relationship between Somatomedin glucose and insulin studied in vivo and in vitro*

Previously we have reported our studies on the relationship between somatomedin (Sm.) HGH and insulin and demonstrated changes in the amount of Sm connected with changes in plasmainsulin The observations were not conclusive and in attempt to clarify these the following studies were done

Insulin tolerance test were performed in 15 patients with various types of growth retardation Glucose insulin IRGH and somatomedin was measured The initial phase was studied particularly since we reported earlier on our findings in the later phase An immediate fall in Sm activity was found in all patients In patients with growth retardation of nonhypopituitary origin this was followed after 20 min by a rapid increase in Sm back to initial values

In hypopituitary patients the fall in Sm continued for the following period and did not return to initial values

Identical variations was found in plasma glucose

The findings reflects true changes of Sm. *in vivo* or altered conditions for the assay in vitro

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ndrolone mean TVI was unchanged at stage $_2$ ($n=9$) significantly ($p<0.01$) diminished t stages P_3 ($n=10$) and P_4 (n9) and sim ar to control values at stage P_5 ($n=5$) There was no difference at BA 11 ($n=9$) but slightly significant ($p<0.05$) decrease in mean TVI at BA 13 ($n=10$) and 14 ($n=6$) At the me of peak height velocity mean TVI was 5 7 cm³ (range 3–3.2 cm³) in the treated group and 10 cm³ (4.5–14.3 cm³) in the control group The difference was significant ($p<0.005$)

When given during puberty oxandrolone de lays testicular development as evaluated by testicular size changes Preliminary results on plasma gonadotropins failed to show signifi cant changes in treated patients Final testic ular size remains to be evaluated

D Knorr F Bidlingmaier O Butenandt & D Engelhardt (München)· *Male pseudohermaphroditism due to 17 β-hydroxysteroid oxidoreductase deficiency in childhood*

A boy with male pseudohermaphroditism was seen at the age of 12 years with marked gynecomastia. Chromosome analysis showed a nor mal male karyotype

Steroid analysis in plasma revealed a tenfold increase of 4-androsten 3 17dione (470–900) ng/100 ml) The plasma testosterone level was in the wide range of early puberty The male pseudohermaphroditism combined with mark edly elevated plasma androstendione suggested a blockage in the androgen pathway behind androstendione

Testicular tissue taken from undescended testes was incubated with ³H-androstendione together with NAD NADH and NADP gener ating systems

In comparison to only 30% of metabolized ³H-androstendione in our patient 92% were found in a control incubation with normal testicular tissue

The turnover rate from ³H-androstendione to testosterone was 55% in the control study but only 15% with testicular tissue of our patient

Plasma oestrone and oestradiol determined radioimmunologically were significantly elev ated oestrone much more than oestradiol in dicating again a blockage of a 17β-hydroxy steroidoxydoreductase

Suppression studies with dexamethasone and fluoxymesterone and stimulation studies with HCG and ACTH suggested that both adrenal glands and testes were involved

B T Rudd P M. Howse P H W Rayner W R Butt M M Smith & G H Holder (Birmingham)· *Hormonal studies in a 4 1/2 year old boy with a unilateral testicular Leydig cell tumour*

A 4 ½ year old virilised boy presented with advanced linear growth (97th percentile) and body weight (90th percentile) B A (7 years) sparse pubic hair (Tanner II) increased penile length (9 cm) and unilateral enlargement of the right testis (Prader 6)

Stimulation with ACTH (?0 IU twice daily for 3 days) produced an increase in urinary steroids by day three Pregnanetriol 1.5 to 4 4 mg/24 hrs 17 OHCS 6 9 to 21 8 mg/24 hrs and 17 oxosteroids 4 6 to 8 1 mg/24 hrs Dexamethasone (2 mg/day 4 days) failed to suppress the urine steroids Small rises in urinary pregnanetriol and 17 oxosteroid occurred after HCG stimulation Plasma studies showed a normal cortisol response to ACTH 12.3 to 34.2 μg/100 ml (Peak response) and good suppression with dexamethasone 15 0 μg/100 ml to 5 0 μg/100 ml Basal plasma LH 6.2 mIU/ml 2nd IRP and FSH <4 mIU/ml 2nd IRP were marginally increased for the age Plasma testosterone was elevated, 94 ng/100 ml and did not rise after ACTH 75 ng/100 ml was unsuppressed by dexamethasone 153 ng/ 100 ml but rose after HCG (1500 units/4 days) to 272 ng/100 ml At operation peripheral blood and spermatic vein Δ4 androstenedione levels were elevated at 400–595 ng/100 ml

existed between post transplant creatinine clearance and SM activity

However in 4 of 5 patients with persisting azotemia of various degree (creatinine clearance 11 8–42 5 ml/min/1 23 m²) subnormal growth continued despite selectively normalized serum SM activity Three of the four poorly growing azotemic patients had the highest average steroid doses in the group (prednisolone >9 0 mg/m²/day) These observations suggest that growth failure in severe chronic azotemia was at least in part due to lack of SM This deficiency is reversible by transplantation After transplantation peripheral antagonism of normalized SM by continued azotemia or by a combination of azotemia and high dosage steroid therapy continued to exist

D B Grant J Hambley D Becker B L Pimstone (Harrow and Cape Town) *Reduced serum sulphation factor activity in 5 children with protein calorie malnutrition*

Arch Dis Childh 48 596 1973

J R Bierich E W Joel & D Schönberg (Tubingen) Role of somatomedin in the effect of hormonal treatment of tall stature

E M Ritzén & F S French (Stockholm) *A high affinity androgen binding protein in testicular efferent duct fluid*

The development of the male internal genital organs from the Wolffian duct the further growth and the function of these organs are completely dependent on androgenic hormones secreted from the testis of the same side (1) The testicular fluid that passes through the efferent ducts into the epididymis contains high concentrations of tesosterone Therefore the epididymis obtains a double supply of androgenic hormones through the blood and through the testicular secretion

In the rat rabbit and ram the efferent duct fluid (EDF) contains high concentrations of a high affinity androgen binding protein (ABP) Rat ABP binds ³H-dihydrotestosterone>³H testosterone>³H 5αpabdristabep3α 17β-diol but does not bind androsterone 3 17-dione When passing through the duct of caput epididymis the rat ABP is partly rabbit ABP completely taken up by the lining epithelial cells or degraded in the lumen ABP has also been identified in the testis and epididymis of monkey and man but has not yet been separated from the similar sex hormone binding protein of human (TeBH) We suggest that ABP serves as a carrier for androgenic hormones from the testis to the androgen dependent male genital ducts thereby protecting the hormone from metabolic degradation and assuring uptake by the lining epithelial cells

C Marti Henneberg A Niiranen O Cachin & R Rappaport (Paris) *Effect of prolonged treatment with oxandrolone on testicular size during puberty in boys with constitutional short stature*

Out of 25 boys with constitutional short stature followed during puberty 10 received oxandrolone (0 10 mg/kg/day) for periods of 2 years to 5 years and 4 months Treatment was started before the children had reached a bone age (BA) of 11 years Fifteen untreated boys were considered as the control group Chronological age (CA) at onset of pubertal growth spurt and peak height velocity as well as the progression of the bone ages in relation to CA and their distribution for a given pubic hair stage (P) were the same in both groups Treated patients and controls were compared for given P stages and BA in order to evaluate changes in testicular volume expressed as testicular volume index (TVI) In patients receiving ox

L. Dutau P Bennet J P Louvet & F ...yard (Toulouse): *Radioimmunoassay of ...iodothyronine in unextracted human plasma*

...asma triiodothyronine (T_3) concentrations ...ve been estimated by a radioimmunoassay ...ing unextracted plasma The immunogen ...ed was prepared by coupling T_3 to B S A ...ith a water-soluble carbodiimide and the anti...odies were produced in rabbits 8-Anilino-...naphtalene sulfonic acid was added in the ...say system to block T_3-TBG binding. Equi...bration was performed at 4°C overnight ...ree and bound fractions were separated using ...extran-coated charcoal The assay procedure ...llowed the measurement of T_3 in 25 to 50 μl ...f plasma, resulting in a sensitivity of 20 ng/...00 ml The mean±S D plasma T_3 in normal ...hildren—12–52 weeks (140 2±34 3 ng/...00 ml $n=17$) 1–9 years (131 5±25 6 ng/100 ml ...$=17$) 9–15 years (141.2±23 8 ng/100 ml $n=$...0)—was not significantly different from the ...nean plasma T_3 in normal young adults ...149 4±17.5 ng/100 ml $n=10$)

R. J Kraaipoel H J Degenhart, H K. A Visser V J M B van Beek P J de Leeuw & J G Leferink (Rotterdam and Eindhoven) *Mechanism of pregnenolone formation from cholesterol Stoichiometry and pathways*

The formation of pregnenolone from cholesterol is one of the most important reactions in steroid-biochemistry The generally accepted scheme of two consecutive hydroxylations (or a concerted O_2 attack) however cannot explain all experimental data.

Using intact bovine adrenal cortex mitochondria, we determined the stoichiometry of the O_2 consumption of the following substrates. 20α-OH cholesterol, 22R-OH cholesterol, 20α,22R-di-OH cholesterol to be 2 2 and 1 mol O_2/mol substrate respectively

Aminoglutethimide-phosphate (Elipten) at a concentration of 40 μg/ml in the same system suppressed the synthesis of pregnenolone from cholesterol completely (≥99%) from 20α-OH cholesterol partially (40%) and from 22R-OH cholesterol not at all (≤1%) With a capillary column gas chromatograph–mass spectrometer combination we identified 20α,22R-di-OH cholesterol as an intermediate of the side-chain cleavage reaction both with 20α-OH cholesterol and 22R-OH cholesterol as a substrate

Chemically prepared Δ^{20-22}-cholesterol (cholesta-5,20(22)-diene 3β-ol) proved to be an excellent substrate for the side-chain cleaving enzyme system Based on these findings we propose a metabolic pathway containing the following sequence of intermediates cholesterol Δ^{20-22}-cholesterol cholesterol-20,22-epoxide (or peroxide) 20α,22R-di-OH cholesterol pregnenolone plus isocaproaldehyde Both 20α-OH cholesterol and 22R-OH cholesterol will split off H_2O to form Δ^{20-22}-cholesterol Preliminary experiments suggest that in short term incubations (15 min) only the aldehyde is formed On the basis of these new findings the enzyme defect present in congenital adrenal lipoid hyperplasia can be located between cholesterol and Δ^{20-22}-cholesterol Elipten probably inhibits enzyme action at the same site

A S Goldman (Philadelphia): *Persistent post pubertal elevation of activity of steroid 5α-reductase in the adrenal of rat pseudohermaphrodites and correction by large doses of testosterone or dihydrotestosterone*

The development of adrenal androgen metabolizing enzymes with androstenedione and testosterone as substrates has been studied in the rat pseudohermaphrodite and its King-X Holtzman male littermates and females The rat pseudohermaphrodite is a genetic male with testes but it has a female phenotype due to a genetic deficiency in target organ androgen receptor protein Enzymatic activity has been determined by the separation and quantitation of the products formed from the labelled androgen substrates by a partition system on thin-layer chromatography and by trimethyl-

exceeding the levels of testosterone A histologically typical Leydig cell tumour was removed

Postoperatively all values were normal with the exception that a slight rebound rise in FSH occurred The high peripheral and spermatic vein levels of Δ4 androstenedione favoured a relative 17 βxydoreductase deficiency in the tumour Plasma studies were more helpful than the urine analyses in deciding the aetiology of the sexual precocity

J Homoki J Birk A T A Fazekas U Loos G Rothenbuchner & W M Teller (Ulm) *Congenital goitre associated with hypothyroidism*

Several investigations have dealt with the important rôle of thyroid hormones for normal brain development during the intra uterine and the perinatal period We studied 22 full term newborns with congenital goitre during their first weeks of life regarding their thyroid function The following determinations were performed plasma TSH PBJ total T_4 iodine TBI and bone age 78 healthy fullterm newborns served as controls Normal ossification of femoral and tibial epiphyses were taken from the literature Newborns with congenital goitre revealed significantly ($p<0\ 001$) lower PBJ ($5\ 65 \pm 4\ 10$ μg/100 ml) and total T_4 iodine ($4\ 28 \pm 3\ 42$ μg/100 ml) levels compared with healthy controls ($9\ 01 \pm 4\ 05$ μg/100 ml and $5\ 77 \pm 2\ 80$ μg/100 ml respectively) TBI did not differ significantly in both groups 13 newborns with goitre lacked ossification of the tibial epiphyses In these patients plasma TSH levels were elevated above 2 S D of the normal mean for age (as high as 470 μU/ml) 9 newborns with goitre revealed a normal ossification as well as a normal TSH level for age (mean 1st day 81 3 2nd day 55 5 3rd day 15 0 from 4th day on 6 5 μU/ml)

During the first weeks of life determinations of PBJ TBI or total T_4 iodine may fail to detect hypothyroidism In contrast single determinations of TSH and X-rays of the knee are sufficient to prove neonatal hypofunction of the thyroid

F Hanefeld I Richter B Weber & S Zabransky (Berlin) *Neurological studies on children with hypothyroidism on long-term treatment*

35 children on long term treatment for hypothyroidism were studied Their age at the time of neurological examination varied from 3 to 20 years

A high proportion (over 50%) showed neurological abnormalities mainly as cerebellar dysfunction Abnormal gait poor coordination athetosis tremor mild ataxia were the main signs More than half showed a history of delayed motor and mental development Delayed and disturbed speech occurred in two thirds of all hypothyroid children

Mental assessment (WISC Stanford Binet) showed 50% of all children with an IQ above 90 According to the onset of symptoms and treatment (0–3 mths 3–6 mths 6 mths and later) we divided our cases in different groups

As for the mental attainment those scored best who received treatment early However there was no such clear correlation between onset of symptoms beginning of treatment and neurological functions in later life A relatively high proportion of early treated children showed neurological abnormalities There was also no clear correlation between IQ motor development and neurological signs However a very low IQ was usually combined with abnormal neurological symptoms delayed speech and retarded motor development

Our findings seem to indicate that in the more severe cases of congenital hypothyroidism cerebral and cerebellar damage occurs already during intra-uterine life

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Plasma triiodothyronine (T_3) concentrations have been estimated by a radioimmunoassay using unextracted plasma The immunogen used was prepared by coupling T_3 to B S A with a water-soluble carbodiimide and the antibodies were produced in rabbits 8-Anilino-1,naphtalene sulfonic acid was added in the assay system to block T_3-TBG binding Equilibration was performed at 4°C overnight Free and bound fractions were separated using dextran-coated charcoal The assay procedure allowed the measurement of T_3 in 25 to 50 μl of plasma, resulting in a sensitivity of 20 ng/100 ml The mean±S D plasma T_3 in normal children—12–52 weeks (140 2±34 3 ng/100 ml $n=17$) 1–9 years (131 5±25 6 ng/100 ml $n=17$) 9–15 years (141 2±23.8 ng/100 ml $n=10$)—was not significantly different from the mean plasma T_3 in normal young adults (149 4±12.5 ng/100 ml $n=10$)

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ilylether derivative formation and radio-gas liquid chromatography The separations have been confirmed by recrystallization Serum corticosterone is significantly higher in the pseudohermaphrodite than in males or females indicating intact corticoidogenic enzymes The development of adrenal androgen metabolizing enzymes in the pseudohermaphrodite differs from that of its littermate male primarily by a persistent postpubertal elevation of activity of 5α-reductase and a failure of a postpubertal rise in 17 ketoreductase the enzyme converting the 17 ketone of androstenedione to the 17β-alcohol of testosterone Development of activity of the enzyme converting the 17β-alcohol of testosterone to the 17 ketone 17β-hydroxysteroid oxidoreductase does not differ in the two animals but differs from that of 17 ketoreductase Postpubertal administration of large doses of androgens correct the persistently elevated levels of 5α-reductase as well as the depressed levels of the 17 ketoreductase in the pseudohermaphrodite to male values The development of these adrenal defects and the correction by postpubertal (but not by prepubertal) testosterone and dihydrotestosterone in the pseudohermaphrodite also occurs in prepubertally orchiectomized genetic male rats

M Stahl J Girard & K Zuppinger (Basle and Bern) *Study of pancreatic glucagon and insulin secretion in children*

A radioimmunoassay specific for pancreatic glucagon was developed The antiserum used in the assay was shown not to cross react with enteroglucagon extracted from porcine gut No glucagon could be detected in plasma samples of a pancreatectomized patient during an oral glucose load At a final antiserum dilution of 1/240000 the assay is sensitive to 60 pg of pancreatic glucagon per ml of plasma

In healthy children aged 3 to 13 years fasting plasma glucagon averaged 110 pg/ml (+52 3 S D) In full-term newborns immediately after birth the mean glucagon concentrations in cord blood was 77 1 pg/ml ($n=21$) In contrast 32 newborns aged 2–12 hours had a mean glucagon level of 811 pg/ml measured in blood samples taken from the prehepatic umbilical vein Glucagon levels were markedly elevated (up to 2000 pg/ml) in 5 diabetic children with ketoacidosis The glucagon concentrations decreased to near normal values within 24 hours after institution of insulin therapy In 15 healthy children aged 3–15 years the infusion of arginine induced a prompt short lived and thee fold elevation in plasma glucagon and an about six fold increase in plasma insulin. Marked hyperresponsiveness of glucagon to arginine infusion was observed in newborns aged 1–6 days In 5 out of 10 children with cystic fibrosis an impaired response of both glucagon and insulin following arginine infusion was found Furthermore the basal concentrations of both hormones tend to be lower in these patients than in normals

M Vanderschueren Lodeweyckx R Wolter P Malvaux E Eggermont & R Eeckels (Bruxelles and Louvain) *Hormonal responses to the administration of glucagon in children*

Plasma growth hormone (GH) immunoreactive insulin (IRI) cortisol and glucose were studied before and after the intramuscular injection of glucagon (0 1 mg/kg) in 53 prepubertal children This group consisted of 11 children with idopathic growth delay 7 coeliac patients 7 children with thyroid disorders 13 hypopituitary patients and 15 children with varied disorders Results were compared with the values observed in normal children Blood samples were taken before and 15 30 45 60 90 120 150 and 180 minutes after the injection of glucagon Minimal side effects were observed in some children GH and IRI were measured by double antibody radioimmunoassay glucose by glucose oxidase method and cortisol by competitive protein binding

Glucose IRI GH and cortisol rose after the injection of glucagon Peak levels were ob-

served at 30 min for glucose and IRI at 120 min for GH and at 180 min for cortisol. The amplitude of the observed reactions was not influenced by age or sex Hypopituitary patients did not show a rise of plasma GH Blunted GH responses were observed in some cases of hypo- or hyperthyroidism obesity coeliac disease and psychosocial deprivation In 30 children either normal or affected by some disorder mentioned above GH response after glucagon could be compared with the response obtained after intravenous insulin With the use of glucagon in the above mentioned dose the maximal GH values attained were as a rule definitely higher than those observed after insulin

Z. Laron Z. Josefsberg & M. Doron (Petach Tikva and Tel Aviv): *Plasma glucagon response to arginine infusion in juveniles with HGH deficiency*

Prolonged HGH deficiency decreases the response of insulin to HGH and causes a tendency to hypoglycemia. It is not known whether pancreatic glucagon is directly or indirectly affected by HGH lack.

The following groups of patients with HGH deficiency were studied

1) Sporadic isolated HGH deficiency (No 8)
2) Hereditary isolated HGH deficiency (No 6)
3) Panhypopituitarism (No 9)
4) Dwarfism with high plasma IR HGH (No 8)

After an overnight fast all subjects were administered a 30 min infusion of 1 arginine (0.5 g/kg and blood samples taken at 0 15 30 60 90 and 120 min for determination of glucagon insulin and glucose Glucagon was determined by a radioimmunoassay system specific for pancreatic glucagon. With two exceptions who revealed high fasting plasma glucagon all fasting levels were within the accepted normal range A variety of responses to the arginine were registered. No direct correlation was found between glucagon and insulin response similar to that we found in children with constitutional growth retardation

P M Howse P H W Rayner B T Rudd & J Williams (Birmingham): *A comparison of growth hormone secretion in children of short stature during nocturnal sleep and insulin induced hypoglycaemia*

Eleven children with short stature (4 5–15 yr) were investigated using a continuous sampling method. Samples for Growth Hormone (GH) and Cortisol were taken at 15 min intervals during nocturnal sleep followed by an Insulin Stimulation Test (IST) the next morning Samples were collected by a heparin-coated Teflon cannula and PVC tubing connected to a Watson Marlow flow inducer Sleep Pattern was monitored by an observer in all and by EEG in some cases

GH peaks showed a relationship to sleep pattern. There was a fair correlation between maximum levels recorded during sleep and following Insulin Induced Hypoglycaemia Individual mean GH secretion in mU/hr has been used as an index of GH production during sleep Group Sleep Means (GSM) indicate mean group (age classified) GH secretion

The 13–15 yr age (9–11 B A.):
GSM=66.6 mU/hr
The 11–13 yr age (7–11 B A)
GSM=35 1 mU/hr
The 9–11 yr age (5–7 B A):
GSM=32 1 mU/hr

The GSM appears to be age related, with a significant increase in the group showing early puberty (13–15 yr) A group with clinical features of hypopituitarism had the lowest GSM of 8 8 mU/hr Two children in this group had peak GH levels during IST of 4 and 6 μU respectively but had sleep peak values of 20 and 25 μU A third child showed a peak GH response of 25 μU to Insulin but a sleep peak of 8 μU Differential hypothalamic re

sponsiveness to sleep as distinct from insulin hypoglycaemia may represent a further category of GH deficiency

M Pierson G Grignon P Nabet Ph Hartemann D Malaprade D Lemoine & F Belleville (Nancy) *Comparative studies of the cytological patterns and secretory activities in human fetal pituitary cultures*

30 foetuses of 7 to 40 weeks of gestational age have been collected and adenohypophysis separated in two parts one for tissue culture the other for histological studies

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(*b*) From the biochemical point of view immuno-reactive HGH and LH could be demonstrated in our 12 explants the youngest one was 7 weeks old

Secretion levels seemed to be produced in a progressive way according to the developmental stage of foetuses but this phenomenon was more evident for HGH than for LH

These results confirm other previous studies as far as HGH is concerned but significantly differ of other investigations about LH activity

Comparison between immuno-reactive secretions and cytological aspects of the same tissue sample give a good correlation for the developmental process of the endocrine function of the human fetal adenohypophysis

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DDAVP dargvasopressin is a new synthetic analogue of the native human antidiuretic hormone It has a specific and long acting antidiuretic effect practically free from pressor effects which has made it very suitable for the treatment of diabetes insipidus

Pitressin in combination with 16 hours thirst has long been used for testing renal concentration function The disadvantages with pitressin are several it has to be injected it is an extract of nonhuman origin and dosage has to be low to avoid pressor effects

It has been the aim of the present investigation to evaluate the hormonal effect on renal concentration capacity

By comparing the effect of DDAVP after intravenous and intranasal application it was found that maximal effect of the hormone could be obtained even after intranasal application

Furthermore the effect of intranasal DDAVP upon urine osmolality was also compared with the outcome of a standard pitressin test on over 30 children of 0–15 years of age With DDAVP drinking was allowed ad libitum The effect of intranasal DDAVP on urine osmolality was found to be equal to the effect of pitressin DDAVP is therefore to be considered a valuable alternative to pitressin in renal function tests

D Schönberg, E W Joel & R. Kappler (Tübingen): *Stimulation of plasma FSH and LH during insulin induced hypoglycemia in pre- and postpubertal children*

In a pilot study plasma FSH and LH were measured by double antibody radioimmunoassay in four prepubertal and six postpubertal dwarfed children during routine insulin tests and in five normal postpubertal children during arginine infusion tests. NIH (Bethesda) materials were used for the assays and the results were expressed in ng LER 907/ml plasma

Prepubertal children mean LH before test 18 7 ng/ml range 16–23 increment LH 7 ng/ml=+37% Mean FSH before test 36 9 ng/ml range 3–74 increment FSH 22.7 ng/ml=+61.5% Mean STH before test 11 7 ng/ml range 3.5–20 mean increment STH 14 4 ng/ml=+123% Mean blood sugar 83 mg% range 76–86 Mean fall 47 mg%=−57%

Postpubertal children mean LH before test 35 6 ng/ml range 19–81 mean increment LH 31 1 ng/ml=+87% Mean FSH before test 97 8 ng/ml range 25–161 mean increment 115 ng/ml=118% Mean STH before test 4.2 ng/ml range 0 9–10.8 mean increment 28 6 ng/ml=+681% Mean blood sugar before test 77 mg% range 63–93 mean fall 34 5 mg%=−45%

Arginine test postpubertal children Mean LH before test 35 2 ng/ml range 19–50· mean increment LH 7 ng/ml=+20% Mean FSH before test 118 8 ng/ml range 33–188 mean increment FSH 7 6 ng/ml=6 4% Mean STH before test 2.5 ng/ml range 1–4 mean increment STH 20.24 ng/ml=+804% Mean blood sugar before test 80.4 mg% range 72–86 mean increment 28 mg%=+35% Mean insulin before test 48 5 μU/ml range 1–96 mean increment insulin 92.8 μU/ml=191%

During hypoglycemia FSH levels are more elevated than LH levels in plasma most pronounced after puberty During arginine infusion in postpubertal children this effect is not seen More detailed studies are under way to show whether insulin induced hypoglycemia is a clinical test of gonadotropin release

J M Limal & A Basmaciogullari (Paris) *Metyrapone test in hypopituitary children*

We have utilized 2 different tests to determine the best stimulation of ACTH applicable in the growth hormone insufficients (arginin-insulin response) 49 children (2–17 years) were given Metyrapone (MP) in a single oral dose at midnight (30 mg/kg) *Short MP test* Among these 49 patients 27 received 3 g/m² oral MP given in 6 doses in 1 day· *Long MP test* A blood sample was taken between 8–8.30 a m before and after MP for dosage of Cortisol (F) and 11 Deoxycortisol (S) by competitive protein binding radioassay

Results (mean values ±S D in μg/100 ml)

S before MP· 1.2±0 7 in patients and 16 normal children. After MP 1) *Short MP test* S=11 9±2.2 (range 8–17 4) in controls and S=11 4±2.1 (range 9–16) in 23/49 patients (normal responders) S reaches only 3 2±1 8 (range 0 2–7 6) in 26/49 children (insufficient response)

2) *Long MP test* S=20 2±3 4 (range 15 2–27) in 10 normal subjects and S=21 1±4 7 (range 15 4–34) in 14/27 patients (normal response) while S reached only 5 4±5 (range 0.2–14 6) in 13/27 patients (low response)

Of the 27 children who had both tests only 3 who did not respond to the short test responded normally to the long test but in these 3 cases the S value after a single dose of MP was borderline In the remaining 24 patients the response was similar in both tests.

Finally we found a very significant correlation (r=0 73 p<0 001) between the value of F before and S after the single oral MP dose which would permit one to define *adrenal reserve* according to the values obtained The short MP test seems to provide an excellent tool for the evaluation of the pituitary reserve of ACTH in children In the unresponsive patients the level of the S response is extremely variable and may suggest differences in the quantitative aspect of the pituitary reserve

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BOOK REVIEWS

J C van Wieringen, F Wafelbakker H P Verbrugge & J H de Haas. Growth diagrams 1965 Netherlands. Wolters-Noordhoff Publishing, Groningen 1971 68 pp.

R W McCammon: *Human growth and development*. Charles C. Thomas Publishers Springfield Ill. 1970. 395 pp US $ 9 00

To be able to evaluate properly the growth status of an individual or a group of children it is necessary to have up-to-date growth standards from "a well-nourished group of the same racial and ethnic background" (WHO). The growth standards must be revised regularly. The reasons are amply described in study from the netherlands where national cross-sectional studies of growth of height–weight and of development of sexual maturation characteristics have been carried out twice in succession in 1955 and 1965. An interval of 10 years for such studies seems to be sufficient at least in industrialized countries. An organization like that in The Netherlands ought to be built up in other countries.

Height–weight was measured on 54 000 individuals (0–4 years), while sexual maturation was assessed on 6 300 individuals (6–25 years). It is worth mentioning that both the build-up of the organization and the methods of measurement are presented in detail in the book. The statistical analysis was performed by means of (per)centiles. But since grossly deviating growth in clinical situations with longitudinal follow up is better evaluated by standard scores (standard deviations), a discussion on that point would have been desirable.

The secular trend had not diminished among the Dutch children and since there were by no means negligible differences between the social classes, it could be expected to continue. Increment in median height had practically ceased at the age of 17 in girls and 18.5 in boys. Adult sexual characteristics were reached by 90 % at the age of 17 in girls and 18 in boys.

Another approach to growth analysis is the prospective longitudinal study. Such studies are on their way in many research centres. The Child Research Council in Denver Colorado USA, has recently published some of their data assembled 1927–1966 on 334 subjects. Most of the book comprises summaries of data analysed cross-sectionally. The longitudinal analyses and the biological interpretations will appear in a later volume. There are presented descriptive statistical summaries of selected segments of physical growth and maturation, health records, nutritional intakes and biochemical and hematological variables. For the anthropometric measurements (per)centiles as well as standard deviations are given. In every section there is a methodological discussion with references to articles with more detailed information.

Even in the mid-1930s the length of this study was extended to include the entire life span in order not to loose potential information. Enrolment in the study has been continuous and is still going on. Of the 334 subjects 179 were being actively followed at the end of 1966. As an attempt to limit the genetic pool preferably first-born children were enrolled and if possible all subsequent siblings. During recent years only second-generation children have been admitted. This attempt might make the biological interpretations of the growth data easier but it also limits the representativity of the material.

John Tarenger

O Neuhäuser: *Folgen encephalitischer Erkrankungen bei Kindern. Untersuchungen zum Problem der sogenannten frühkindlichen Hirnschädigung*. In Vivell & Bernsheiter (eds.) Bücherei des Pädiaters Heft 67. Ferdinand Enke Verlag, Stuttgart 1972 96 pp illus DM 34.–

The authors presents a comprehensive study of a group of children treated for encephalitis in the pediatric department of the University Clinic in Erlangen-Nürnberg during the years 1956–70. The series consisted of 286 children with a mortality of 29 %. The children 5 to 14 years of age were chosen for a closer investigation. 81.6 % (91 children) of those were examined neuro-psychiatrically. Some rather new tests for detecting minor defects in motor coordination and perceptual function are described.

The course of the acute encephalitis and the early history of the patients were evaluated and correlated to mortality and symptoms of brain damage. Statistical valuation of a material of this type is difficult. The author found, however, that the time factor and the occurrence of seizures were the only sure prognostic signs. Encephalitis was more prevalent in infancy and so was mortality and grave defect syndromes.

Thirty percent of the children described as recovered had some minor signs of motor incoordination and perceptual difficulties. The author stresses the fact that all children who had encephalitis must be

READ BY TITLE

B Betend L David & R Francois (Lyon) *Familial hypogonadotrophinuric hypogonadism with resistance to treatment with H C G*

C Dacou Voutetakis C Theodoridis A Agathopoulos & N Constantinidis (Athens) *Nychthemeral variation of plasma growth hormone in diabetic children*

A T A Fazekas J Homoki S B Pal & W M Teller (Ulm) *Relations of body weight and surface area of C_{21} and C_{19} steroid excretion in newborns infants and children*

C C Forsyth (Dundee) *A review of fetal and neonatal adrenocortical function*

C C Forsyth & J Cameron (Dundee) *Adrenocortical function in myotonic dystrophy of childhood*

C C Forsyth (Dundee) *Follow-up of two boys treated with cyproterone acetate*

H Gleispach J J Alcaniz Ferrando B Barcelo Lucerga L S Hernandez & J Glatzl (Innsbruck and Madrid) *Changes in the urinary steroid pattern of a 4 1/2 year old boy with a tumour of the adrenals*

R Illig S Pluznik C G D Brook & A Prader (Zürich) *The TRH test in children with hypothalamic and pituitary disorders*

M Karp Z Laron & M Doron (Tel Aviv) *Dynamics of insulin and glucagon responses in children with constitutional familial short stature*

K Klossoglou C Theodoridis A Nicolaidis T Karpathios & Matsaniotis (Athens) *Leukocyte alkaline phosphatase activity in congenital hypothyroidism*

M Koivisto & H K Åkerblom introduced by Hilkka Hiekkala (Oulu and Helsinki) *Hypothyroidism and growth hormone deficiency in a boy having a deletion of the long arm of chromosome No 1 (46 XY lq–)*

B M Laurance B O Connel & A Robinson (London) *Parameters for monitoring growth in children with congenital adrenal hyperplasia*

P W Nars K K Dighe W M Hunter & J Girard (Basle and Edinburgh) *Measurement of steroid hormones by radioimmunoassay techniques*

K E Petersen & M Damkjaer Nielsen (Glostrup and Copenhagen) *Difficulties in the exact biochemical diagnosis in congenital adrenal hyperplasia in the neonatal period*

W v Petrykowski & P Burmeister introduced by W Teller (Freiburg) *A family with hereditary adrenocortical unresponsiveness to ACTH*

P H W Rayner & B T Rudd (Birmingham): *An assessment of the value of cyproterone acetate in the retardation of skeletal development in sexual precocity in boys*

H Schedewie U Heinrich K E von Mühlendahl & H Helge (Heidelberg and Berlin) *Endocrinological and neurological studies in a girl treated over 2 years with OP DDD because of relapsing virilizing adrenal carcinoma*

D Schönberg E W Joel W Ilg R Kappler & E Jetter (Tübingen) *Individual patterns in circadian rhythms of plasma growth hormone FSH and LH*

V Stanescu R Stanescu & P Maroteaux (Paris) *Studies on the proteoglycans of the growing cartilage*

A Van Steirteghem R Leclercq M Bossuyt R Wolter & J R M Franckson (Lwiro and Brussels) *Study of the hypothalamo-pituitary-adrenal axis in marasmic kwashiorkor*

H Stolecke & H A Hienz (Krefeld) *Testicular tissue in intersexuality—response to HCG clinical and histological examinations*

C G Theodoridis C Dacou Voutetakis M Constantinidis & T Karpathios (Athens) *Fasting growth hormone levels in pre-pubertal and pubertal children*

S Zabransky & A v zur Mühlen (Göttingen and Berlin) *Influence of dextro-triiodothyronine (dT3) on thyrotropin (TSH) secretion in men*

Acta Paediatr Scand 63 341–346 1974

PHOTOSENSITIZED SHIFT IN THE O_2 DISSOCIATION CURVE OF FETAL BLOOD

ENRIQUE M OSTREA, JR. and GERARD B. ODELL

From the Department of Pediatrics Johns Hopkins University School of Medicine and the Harriet Lane Service of the Children Medical and Surgical Center of the Johns Hopkins Hospital Baltimore Maryland USA

ABSTRACT Ostrea, E. Jr., and Odell, G (Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA). Photosensitized shift in the O_2 dissociation curve of fetal blood. Acta Paediatr Scand 63: 341 1974.—The exposure of fetal erythrocytes to blue light in the presence of bilirubin was associated with a significant decrease in the affinity of fetal cells for O_2, (ΔP_{50}) at pH 7.4 = +3.17 ± 0.64 mmHg. This change was not observed with either adult erythrocytes or hemolysates of fetal and adult red cells. Associated with the shift in P_{50}, there was a decrease in the Na K -ATPase of the irradiated erythrocytes and no electrophoretic evidence of alteration in the fetal hemoglobin. The results suggest that the shift in the O_2 dissociation curve represents an additional manifestation of photodynamic membrane injury and the stroma of fetal erythrocytes influence their oxygen affinity

KEY WORDS: Bilirubin, blood O_2 dissociation, photosensitized hemolysis, phototherapy

The extensive use of phototherapy in the management of neonatal hyperbilirubinemia has led to reports of possible adverse effects of intensive exposure of newborns to the radiant energy within the visible spectrum (2 12) This laboratory recently reported hemolysis of red cell suspensions in vitro (17) when irradiated by the phototherapy units currently used clinically The hemolysis required the simultaneous presence of oxygen light and bilirubin and thereby demonstrated that bilirubin was a photosensitizing agent and had photodynamic action.

This report records the observation that human fetal cells reduce their affinity for oxygen near to that of adult erythrocytes after brief irradiation with blue light in the presence of bilirubin This shift in the oxygen dissociation curve was observed only in intact fetal erythrocytes and was always associated with evidence of membrane injury reflected by a loss in the cation sensitive membrane Na^+ K ATP ase

These studies were supported by U.S Public Health Service Grant HD-02268 from NICHD

Doctor Ostrea s present address is The Hutzel Hospital, Detroit, Michigan.

MATERIALS AND METHODS

Four types of human fetal red cell-bilirubin preparations were studied.

(A) Red cell-bilirubin suspensions: Placental cord blood of newborn infants was collected and centrifuged to remove the plasma layer The packed cells were washed once in 3 volumes of 0 15 M NaCl and re-suspended in isotonic 0.1 M Tris-PO buffer (pH=8.0 at 25°C) to a final hematocrit of 43–45% Bilirubin (Pfanstiehl Lab) was dissolved in N_2-gassed 0 025 N

followed with special training of these functions in order to achieve the best results and avoid psychiatric and school problems

It is very difficult to find good surveys about late prognosis in encephalitis and I think the author has contributed with an interesting examination with answer to at least some of the questions He also gives a good view of the complexity of the problem due to many different factors influencing the outcome in childhood encephalitis i e type of infectious agent age and premorbid status of the child

Ingrid Bjerre

Allen W Root *Human pituitary growth hormone* Charles C Thomas Springfield Illinois 1972 259 pp illus US $ 10.50

There has been a dramatic increase in our information concerning pituitary growth hormone during the last decade In this monograph the biochemical, physiological and clinical aspects of this important pituitary principle are reviewed. In the first section of the book there is a detailed discussion of the chemistry and immunology of human growth hormone and thereafter its effects upon protein lipid carbohydrate and mineral metabolism are presented. The following chapter deals with methods for the measurements of growth hormone in biological fluids and the neuroendocrine control of growth hormone secretion. The various provocative stimuli for growth hormone secretion are described and its secretion in a variety of clinical states including diabetes mellitus adrenocorticoid excess and obesity is reviewed.

The etiology and diagnosis of hypopituitarism are *extensively discussed and the results of therapy with* human growth hormone in large series are presented. A chapter is devoted to the description of clinical and laboratory findings in patients with acromegaly and to modes of therapy for this condition

In the last part of the monograph the author summarizes the most recent progress in the field of human pituitary growth hormone

The book is very comprehensive and well illustrated Inasmuch as over seven hundred references are included this book will also serve as a ready reference source

Karl Olof Nilsson

ANNOUNCEMENTS

The annual meetings of the American Pediatric Society the Society for Pediatric Research and the Ambulatory Pediatric Association will be held at the Sheraton Park Hotel, Washington D C April 29 through May 3 1974 The schedule for these meetings is as follows

Monday April 29 a.m. & p m Ambulatory Pediatric Association
Tuesday April 30 a.m & p m Ambulatory Pediatric Association
Wednesday May 1 a.m American Pediatric Society (Symposium)
Wednesday May 1 p m American Pediatric Society (Plenary Session)
Thursday May 2, a.m. American Pediatric Society & Society for Pediatric Research (Subspecialty Sessions)
Thursday May 2, p m Society for Pediatric Research (Plenary Session)
Friday May 3 a.m & p m American Pediatric Society & Society for Pediatric Research – Subspecialty Sessions)

The combined registration fee (APS SPR & APA) is $15 The APS & SPR Program and Abstracts will be *available after April 15th from Charles U Lowe* M D Editor-in-Chief Pediatric Research National Institutes of Health Building 31 Room 3A 51 Bethesda, Maryland 20014 The APA Program and Abstracts will be available after April 15th from E. S Hillman M D (address below) For additional information write to Charles D Cook, M D (Secretary American Pediatric Society 333 Cedar Street New Haven, Connecticut 06510) Jo Anne Brasel, M D (Secretary Society for Pediatric Research Columbia University College of Physicians & Surgeons 630 West 168th Street, New York, New York 10032) or Elizabeth S Hillman, M D (Secretary Ambulatory Pediatric Association 2300 Tupper Street Montreal Quebec Canada).

The 1974 Birth Defects Conference will be held at the Newporter Inn, Newport Beach California from June 16–20 1974 under the joint sponsorship of The Harbor General Hospital Campus of the UCLA School of Medicine and The National Foundation-March of Dimes. Topics for discussion will include Heritable Disorders of Connective Tissue Epiphyseal Dysplasias Mucolipodoses New Malformation Syndromes, Hypogonadism and Genital Tract Malformation Syndromes New Chromosomal Syndromes and Fetal Visualization and Sampling Techniques (fetoscopy) Further information can be obtained by writing to David L. Rimoin M D Ph D Harbor General Hospital 1000 West Carson Street Torrance California 90509

Table 1 *Effect on the P_{50} of the exposure of fetal red cells to blue light in the presence of bilirubin*

Fetal red cell-bilirubin preparation[a]	Exposure time[b]	P_{50} (mmHG) at 37°C, pH=7 4 Light protected	Light exposed	ΔP_{50} (mmHg)
A. Red cell-bilirubin suspension (2 mg/100 ml bilirubin)	15 min 10 min	18.5 20.5	21.0 24 0	+2.5 +3.5
B Red cell-bilirubin albumin suspension (1 1 bilirubin-albumin molar ratio 15 mg/100 ml bilirubin)	6 hours	23.5	27 0	+3.5
C. Cord blood-bilirubin mixture (20 mg/100 ml bilirubin)	12 hours 11 hours 10 hours	20.0 23.5 22.5	24.2 27.0 24.5	+4.2 +3.5 +2.0
D Pre-exchange infant blood (24 mg/100 ml bilirubin)	5 hours	22.5	24.5	+2.0
Mean S.D				=+3 17 =±0 68 p<0 001

[a] For details, see text.
[b] The exposure time is an arbitrarily chosen value and does not imply the minimum period of light exposure to achieve the desired effect.

To demonstrate the dependence of decrease in oxygen affinity on both light and bilirubin a comparison was made of the O_2 dissociation curve of whole fetal blood when exposed to light in the presence and absence of bilirubin (Table 3) Sufficient bilirubin was added to cord blood to exceed the albumin binding capacity and permit the excess bilirubin to be taken up by the red cell. In the absence of light, no shift of the P_{50} was observed (Samples 1 and 3) In the presence of light and bilirubin, a 4.3 mmHg. increase in P_{50} occurred *(Sample 4) despite the decrease in* 2,3 DPG concentration. The slight increase in the P_{50} of the control sample exposed to light (Sample 2) is consistent with the interpretation that whole blood may contain other substances that can act as photosensitizing agents (7)

In order to distinguish a direct effect of the radiant energy on fetal hemoglobin from an indirect effect on its erythrocyte stroma, two additional studies were done

1 Fetal hemoglobin solutions were prepared by freeze-thaw exposure of packed cells from placental cord blood. The hemolysates were diluted in 0 1 M Tris-phosphate buffer (pH = 8 0 at 25°C) to a concentration of hemo*globin of 10.2 g/100 ml The stroma was re*moved by centrifugation at 48000 g for 15 min. To the supernatant hemoglobin solution, 2,3 DPG and bilirubin were added to yield

Table 2 *Effect of the 10-min exposure of fetal red cell suspension to blue light in the presence of bilirubin (2 mg/100 ml)*

	P_{50} at 37°C & pH=7 4 (mmHg)	2,3 DPG (mM/l)	Decrease of membrane ATP'ase activity (%) Na K	Mg++	Increase in methemoglobin (%)	Supernatant potassium (mEq/l)	Supernatant hemoglobin (mg/100 ml)
Light protected	20.5	3 0	–	–	0	2.46	200
Light exposed	24 0	2.9	15	5	0	3 97	240

NaOH and added to the cell suspension to yield a calculated concentration of 2 mg/100 ml in the extracellular phase [1]

(B) Red cell-bilirubin-albumin suspensions. The red cells from cord blood were isolated and washed as above and resuspended in 0.01 M PO_4 buffer (pH=8 0 at 25°C) containing 0 9% NaCl 100 mg/100 ml dextrose and ? g/100 ml crystalline human albumin Bilirubin prepared as in (A) was added to yield a 1 1 molar ratio with respect to albumin

(C) Cord blood-bilirubin mixtures. Bilirubin prepared as in (A) was directly added to placental cord blood to yield a serum concentration of ?0 mg/100 ml

(D) Pre-exchange newborn blood Blood was obtained from jaundiced infants prior to exchange transfusion

From each red cell-bilirubin preparation 10 ml aliquots were pipetted into rectangular tissue culture flasks (Falcon Plastic 3024 Tissue Culture Flask with a surface area of 75 cm^2) Half of the flasks were protected from the light by wrapping them in aluminum foil All of the flasks were horizontally rotated (Multi Purpose Rotator Model 150B Scientific Industries Inc.) in a dark room at a constant temperature of 36.5°C for 10 minutes. After temperature equilibration the flasks were exposed to the blue light of a Bili Lamp® (Olympic Surgical Company GE F20T 1?-B) which gave a mean incident radiation of 9 6 mwatts/cm^2 in the 420–480 nm spectral range The shortest exposure time was 10 min (range from 10 min to 12 hours)

The oxygen dissociation curve of the cell suspensions were determined by equilibration of 0 8 ml of blood in the tonometers of the Astrup blood gas machine (Radiometer) for 15 min All the equilibrating gas mixtures were humidified and contained 4% CO_2 with varying concentrations of oxygen and nitrogen The pH and Po_2 of the equilibrated blood samples were measured in a capillary glass electrode (Radiometer Type G297) and a Po_2 electrode (Radiometer Type E5046) respectively The O_2 saturation was determined spectrophotometrically (Zeiss Spectrophotometer PM Q-II) by the method of Dubowski (9) The Bohr factor was applied to convert each result to its corresponding value at pH=7 4 (22) A minimum of four different oxygen concentrations were used The sigmoidal O_2 dissociation curve was transformed to a linear graph by plotting log $SO_2/(100-SO_2)$ against the log Po_2. The Po_2 of 50% oxygen-saturation of the blood (P_{50}) was interpolated from the graph and the differences in values (ΔP_{50}) were compared in the light-protected samples The concentrations of methemoglobin (16) membrane ATP ases (10) 2,3 diphosphoglycerate (2,3 DPG) (14) and immediate (light) losses of potassium and hemoglobin from the erythrocytes (17) were measured in the preparations.

[1] The measured concentration of bilirubin in the cell-free media was less than 0.05 mg/100 ml because the erythrocytes seqestered most of the added bilirubin.

RESULTS

A summary of the shift in oxygen dissociation curves (ΔP_{50}) is recorded in Table 1 *In all of the fetal red cell suspensions* the in vitro exposure of fetal erythrocytes to blue light in the presence of bilirubin was associated with a significant decrease in the affinity of fetal cells for oxygen when compared to the control samples protected from light Mean Δ P_{50} at pH 7 4=+3 17±0 68 mmHg An example of the effect of irradiation of fetal erythrocyte suspensions in protein free media is summarized in Table 2 The data illustrate that the brief exposure of fetal red cells to blue light in the presence of bilirubin was associated with a mean increase of 3 5 mmHg in the P_{50} of the irradiated samples and evidence of erythrocyte membrane damage The latter manifested as a 15% loss of Na^+ K^+ membrane ATP ase with only a 5% decrease in the magnesium ATP ase and a greater immediate potassium and hemoglobin leak from the irradiated red cells The concentrations of 2,3 DPG and methemoglobin were similar in the control and irradiated samples In three similar experiments the irradiated samples showed Losses of Na^+ K^+ ATP ase that varied between 10 and 28% and decreases in the Mg^{++} ATP ase of 0 to 7% the concentrations of 2,3 DPG remained unchanged or decreased by 0 2 to 0 5 mM/l the immediate potassium and hemoglobin leaks were consistently greater and the methemoglobin concentrations were unchanged or increased

Irradiation of erythrocyte suspensions prepared from the blood of normal adults was associated with the same decrease in cation sensitive ATP ase and the accelerated loss of potassium and hemoglobin from the irradiated erythrocytes with similar changes in the concentrations of methemoglobin or 2 3 DPG as described above In contrast to fetal erythrocyte suspensions no change in the P_{50} was observed in irradiated suspensions of adult erythrocytes

served only with irradiation of fetal erythrocytes either in whole blood or in suspensions, but not with adult cells or the hemoglobin solutions Intact preexchange blood with an elevated serum bilirubin saturation index (>8) also manifested a similar shift in the P_{50} of its O_2 dissociation curve This preliminary observation is consistent with the previous studies that high bilirubin saturations of albumin are associated with significant concentrations of bilirubin in the circulating red cells (24) The results suggest that if phototherapy were applied to jaundiced infants with a high saturation of their albumin with bilirubin the potential for a light induced hemolytic reaction and a reduction in O_2 affinity of the red cells is enhanced Such an in-vivo occurrence would require sufficient transmission of incident light to the capillary circulation

The oxygen dissociation curve of blood is known to be affected by a variety of factors Elevation in temperature hydrogen ion concentration, P_{CO_2} and ionic strength are associated with a decrease in the affinity of hemoglobin for oxygen and a shift in the oxygen dissociation curve to the right. Since both the irradiated and control samples were equilibrated in the same gases at the same pH and temperature the above factors would not explain the shift.

More recently it has been demonstrated that the concentration of organic phosphates within the erythrocytes particularly 2,3 DPG and ATP reciprocally influence the affinity of hemoglobin for oxygen (4 6) In humans although the level of 2,3 DPG is the same in normal fetal and adult red cells (25) their difference in oxygen affinity has in part been explained as due to the weaker binding of 2,3 DPG to fetal hemoglobin (8 23). One possible explanation for the observed ΔP_{50} which we examined was the possibility of a light induced alteration in the structure of the fetal hemoglobin in the intact erythrocyte. The resultant product might be associated with a decreased oxygen affinity either through a change in the tertiary structure or increased binding of 2,3 DPG However photosensitized oxygenations of proteins usually affect the aromatic amino acids and result in the formation of more negatively charged molecules (11). Such an effect would be expected to further retard ionic attraction for the negatively charged 2,3 DPG and result in a more rapid electrophoretic migration of the hemoglobin and its polypeptide chains The absence of any change in the electrophoretic behavior of the hemoglobins and their polypeptide chains indicated no significant change in surface charges had occurred in the irradiated samples It is conceivable that the peptide chains could have been altered without affecting their surface charge Such a change may not have been detected by the electrophoretic techniques applied in the present report and would require a more detailed analysis of the peptide chains. However other studies have suggested that the cell membrane and stroma of the fetal erythrocyte also influence its oxygen affinity (1 3 21) The present results showed that the reduction in affinity of red cells for O_2 was observed only in the intact fetal erythrocytes and always in association with evidences of membrane injury This suggests that the shift in P_{50} is more likely a manifestation of photodynamic membrane injury and supports the interpretation that the membrane of fetal erythrocytes may contribute a significant role in determining the affinity of fetal red cells for oxygen

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Table 3 *The P_{50} of fetal whole blood after 10-hour exposure to blue light with and without added bilirubin*

	Serum bilirubin (mg/100 ml)	P_{50}(mmHg) at 37°C pH=7 25	2,3 DPG (mM/l)	Methemoglobin (g/100 ml)
Control				
1 Light protected	0.5	20 2	1 96	0 100
2 Light exposed	0.5	21 8	1 96	0 180
Bilirubin added				
3 Light protected	17.8	20 2	2.34	0 080
4 Light exposed	17.8	24.5	1 43	0.210

final concentrations of 1 5 μMol DPG/g Hb and 2 mg/100 ml respectively These samples after irradiation had the same P_{50} as the light protected control solutions (11 mmHg) The α β and γ chains were isolated from these hemoglobin solutions and no differences were detected in the electrophoretic mobilities of the polypeptide chains between irradiated and controls (13)

2 Fetal erythrocytes were prepared as described for suspensions A of Table 1 and irradiated to produce a significant ΔP_{50} The hemoglobins from the irradiated and control suspensions were analyzed simultaneously by starch gel electrophoresis and the migration patterns of the control and irradiated hemoglobins were identical

The role of bilirubin as a photosensitizing agent is well documented (15 17) However to act on the red cell membrane bilirubin like other photosensitizing agents has to be attached to the membrane (5 17) Normally bilirubin in the blood is preferentially bound to serum albumin and only after saturation of the albumin will bilirubin be present in red cells at significant concentrations (18 24) The determination of the relative saturation of serum albumin with bilirubin has been reported by Odell et al and the saturation index can vary between 0–14% (19)

Blood with known saturation indices was collected from two infants with hyperbilirubinemia at the time of exchange transfusion and exposed in vitro to blue light The ΔP_{50} of the irradiated samples in comparison to the controls is shown in Table 4 The results illustrate that Patient 1 who had a low saturation index did not show a shift in P_{50} despite a high serum bilirubin concentration, whereas Patient 2 who had a high saturation index demonstrated a significant increase in P_{50} despite a lower serum bilirubin concentration

DISCUSSION

The previously demonstrated photosensitized hemolysis of erythrocytes by bilirubin were again observed in the present studies (17 20) The photodynamic action of bilirubin on red cells was characterized by a significant decrease in the membrane Na^+ K^+ ATP ase an accelerated cation loss of potassium from the red cells and eventual colloid osmotic hemolysis In addition a significant reduction in the affinity of fetal red cells for O_2 was found in association with membrane injury The decrease in the oxygen affinity was ob-

Table 4 *Effect of blue light on the P_{50} of whole blood from jaundiced infants with different degrees of serum saturation with bilirubin*

	Serum bilirubin (mg/100 ml)	Saturation index* (%)	ΔP_{50} (mmHg)
Patient 1	23 6	4.5	0
Patient 2	17 6	8.5	+2.5

* A saturation index of 8 or above indicates a high saturation of serum albumin (19).

Acta Pædiatr Scand 63 347–350 1974

CALCIUM AND PHOSPHORUS CONTENT OF TRANSITIONAL AND MATURE HUMAN MILK

D. BARLTROP and R. HILLIER

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ABSTRACT Barltrop, D. and Hillier R. (Paediatric Unit, St. Mary's Hospital Medical School, London, England) Calcium and phosphorus content of transitional and mature human milk. Acta Paediatr Scand 63: 347 1974.—The mineral content of breast milk is known to vary but the significance of this for the newborn is unknown. The calcium and phosphorus content of breast milk from 58 nursing mothers has been determined at intervals up to 6 weeks *post partum*. The plasma calcium and phosphorus content of 13 of the infants aged 6 days was also measured. The calcium and phosphorus content of transitional milk increased during the first 6 days of lactation without significant alteration in Ca/P ratio. No relationship between milk composition and infant plasma chemistry could be demonstrated at the 6th day. The data suggest that milk Ca/P ratios are of less significance for neonatal calcium homeostasis at low as opposed to high mineral loads.

KEY WORDS: Breast milk, composition, Ca/P ratio, calcium homeostasis

Breast milk is commonly assumed to be ideal for the newborn infant and increasingly attempts have been made to create infant formulae which conform with its composition For comparative purposes pooled mature breast milk is usually accepted as the reference material and little account is taken of the variations that occur during the first 10 days of lactation. Thus colostrum and transitional milk differ from mature breast milk in the lesser volume that is available and in relatively low calorie and mineral content (1 2) Variation may also occur in an individual milk at different feeds and between breasts during a particular feed (3) Although cows milk formulae differ in composition the infant fed with a given preparation receives feeds which have been manufactured to be of almost constant composition

The biological significance of these variations has been little studied with respect to neonatal mineral metabolism although they are clearly important in defining the limits to which breast fed infants have to adapt. Although neonatal hypocalcaemia is rare in the breast fed infant (4) it is known that wide variations in the plasma calcium can occur in infants fed both human milk and cows milk formulae There is evidence that one factor influencing the concentration of calcium in the plasma of the newborn is the Ca/P ratio of the ingested milk (5) but little is known concerning this ratio in individual as opposed to pooled breast milks. Although there is general agreement that milks of low mineral content are advantageous for the neonate there is little information concerning the plasma calcium response to variations in the Ca/P ratio at low mineral loads In this paper the calcium and phosphorus contents of a series of individual transitional and mature breast milks are reported The plasma

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Table 3 *Coefficients of variation (S.D /Mean %)*

	Transitional	Mature
Calcium	23.9	19.9
Phosphorus	29.7	18.5
Ca/P	25.0	27.8

2). The phosphorus content of the milks increased at a greater rate than the calcium contents so that the regression line for the Ca/P ratios decreased from 2.2 at 26 hours to 1.6 at 140 hours however the variability of the data was such that this relationship was not significant.

Comparison of the data for transitional milk with mature milk from 12 mothers showed that there was an insignificant decrease in mean calcium content and in Ca/P ratio during the interval between the 1st and 6th weeks of lactation. The mean phosphorus content of the milk increased during the same interval

The mean calcium, phosporus and Ca/P ratios for both transitional and mature milks are given in Tables 1 and 2 together with data collected from the literature The wide range of mineral contents and ratios at both stages of lactation is reflected in the standard deviations about the mean and the correspondingly great coefficients of variation (Table 3)

The mean plasma calcium and phosphorus concentrations in 15 breast fed infants at the 6th day of life were consistent with those previously reported The mean plasma calcium was 10.1±0.9 mg/100 ml and the mean plasma inorganic phosphorus 6.4±0.6 mg/100 ml. Comparison with the corresponding Ca/P ratio for the breast milk secreted at the sixth day indicated a tendency for the plasma calcium to increase and the plasma inorganic phosphorus to decrease as the Ca/P ratio of the breast milk increased (Fig. 3) In this group the breast milk Ca/P ratios ranged from 1.1–2.3 The relationships between plasma chemistry and Ca/P ratio of breast milk were not statistically significant for plasma phosphorus and attained a significance of only 0.1 for plasma calcium

DISCUSSION

The data for the calcium content of breast milks reported in this paper differ from the values given by Macy 1949 (6) but are in general agreement with more recent work (7 9 10) This may reflect differences in analytical techniques for the determination of calcium and it is of interest that the data for phosphorus are in agreement with previous reported values The mean Ca/P ratio for mature breast milks of 1.8±0.1 is lower than the generally accepted value although Widdowson reported values of 1.85 for transitional milk and 2.03 for mature milk from three mothers (11) By comparison cows milk has a Ca/P ratio of approximately 1.3 although the total mineral content is 3–4 times greater than that of breast milk

Although the importance of the Ca/P ratio has been emphasised in connection with neonatal homoeostasis in full term infants fed cows milk the relationship would not seem to have been previously explored at low mineral loads The lack of any statistically significant correlation between calcium and phosphorus contents of plasma and the cor

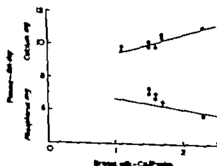


Fig 3 Plasma calcium and inorganic phosphorus concentrations from 15 full-term breast-fed infants at the 6th day of life with regression lines compared with the Ca/P ratio of the corresponding breast milk at the 6th day of lactation.

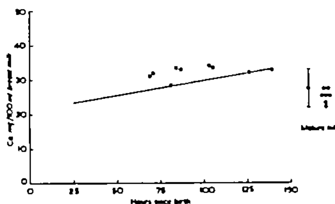


Fig 1 Calcium content of transitional milk from 31 mothers during the first 6 days of lactation with regression line compared with the calcium content of mature milk from 17 mothers at the 6th week of lactation together with mean and S D

Table 1 *Calcium and Phosphorus content of transitional breast milk*

Author	Calcium (mg/100 ml)	Phosphorus (mg/100 ml)	Ca/P
Macy 1949 (6)	46 4±9.5	19.8±4 7	2.3
Hanna et al 1970 (7)	26.2	18.2	1 4
Barnett, 1968 (8)	33	18	1.8
Prinsloo et al 1970* (9)	29 3±6.9	16 4±4 1	1.8
This paper	28.0±6.7	15.5±4 6	2.0±0.5ᵃ

Data calculated from mineral contents/100 g dry matter for white mothers assuming total solids of 13.3 g/100 ml milk (5)
ᵃ Mean and S D of the Ca/P ratios from individual milk samples.

calcium and phosphorus of breast fed infants at the sixth day of life have been determined and related to the composition of their mother s milk

METHODS

Milk samples were obtained consecutively from nursing mothers who had delivered in hospital Three groups were studied at intervals after parturition

Group	Number	Time post partum	Specimen
I	31	26–140 hours	Milk
II	15	6 days	Milk+Infant plasma
III	17	6 weeks	Milk

In each case a 2.0 ml specimen of milk was obtained from one breast at the beginning of the midday feed

Samples of capillary blood were obtained by heel prick from the infants of mothers in Group II at the time of obtaining the milk sample and collected into lithium heparin tubes

The specimens of milk were prepared for analysis by ashing duplicate aliquots in silica crucibles at 500°C in a muffle furnace. To minimize losses, 2–3 mg of barium carbonate was added to each crucible as a carrier before ashing. After ashing, the milk residues were dissolved in 1% HCl for analysis but specimens of plasma were analysed directly after dilution with 0.78% disodium EDTA Calcium determinations were made by atomic absorption spectroscopy and inorganic phosphorus with a conventional molybdate/vanadate method.

RESULTS

The calcium and phosphorus content of transitional breast milks in 31 mothers was found to increase progressively in the interval 26–14 hours after parturition The relationship was linear with time and the correlation coefficient was in each case significant (calcium $p = 0\ 01$ phosphorus $p = 0\ 001$) (Fig. 1

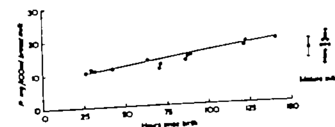


Fig 2 Phosphorus content of transitional milk from 31 mothers during the first 6 days of lactation with regression line compared with phosphorus content of mature milk from 12 mothers at the 6th week of lactation together with mean and S.D

Table 2 *Calcium and phosphorus content of mature breast milk*

Author	Calcium (mg/100 ml)	Phosphorus (mg/100 ml)	Ca/P
Macy 1949 (6)	34 4±6.7	14 1±2.5	2.4
Lough et al. 1960* (10)	25 6±7 4	–	–
Barnett, 1969 (8)	35	15	2.3
Hanna et al 1970 (7)	20 7	18.2	1 7
This paper	27 6±5 5	16.2±3 0	1 8±0.5ᵃ

Derived values from published data.
ᵃ Mean and S D of the Ca/P ratios from individual milk samples

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AN ADDITIVE METHOD FOR AIRWAY RESISTANCE MEASUREMENT

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ABSTRACT Kurel, H. (Physiological Laboratory of the Paediatric Clinic, Karolinska Hospital, Stockholm, Sweden and the Research Institute for Child Development, Charles University Prague, Czechoslovakia). An additive method for airway resistance measurement. Acta Paediatr Scand, 63: 351 1974.—In order to increase the possibilities of determining the airway resistance especially in children, an additive method has been developed which does not require cooperation of the subject being examined nor estimation of alveolar pressure. By means of mathematical derivations it is shown that the ratio mouth pressure difference and flow difference at instant change of additive resistances gives the airway resistance in laminar as well as nonlaminar flow. Besides a recording unit, the equipment consists of a special pick-up tube with three parts: a flow pick-up, a mouth pressure pick-up and two added resistances with means for alternating between them. The method has been tested by using artificial flow resistances and by means of a conventional body plethysmographic method in 4 healthy adults, 10 healthy children and 8 asthmatic children. A correspondance within ±4% of obtained values was found with no significant differences. The method does not call for any artificial breathing manoeuvre, does not cause any unpleasant sensation and allows repetition at short intervals.

KEY WORDS: Airway resistance, children

Classic methods of airway resistance measurement, i.e. the body plethysmographic method and the method of air flow interruption still suffer from some disadvantages in routine use. The former needs the cooperation of the subject being examined and the necessary equipment is expensive while the latter does not seem to yield correct results. Attempts have therefore been made to develop another method which would eliminate these disadvantages and would not require alveolar pressure estimation which is the cause of most troubles. One such attempt is the additive method the theory apparatus and use of which are presented in this paper

This work was supported by the Swedish National Association for Heart and Chest Diseases.

The basic relation from which the airway resistance is derived is:

$$P_{alv} = R_{aw}\dot{V} \qquad (1)$$

where P_{alv} = alveolar pressure as a pressure difference between alveoli and ambient atmosphere, representing a driving pressure across the airway resistance R_{aw} = the airway resistance $\dot{V}$ = volume rate of change.

The airway resistance is then expressed:

$$R_{aw} = P_{alv}/\dot{V} \qquad (2)$$

The relation between alveolar pressure and flow in human airways was found to be nonlinear. Therefore the airway resistance value of a bronchial tree is not constant but changes with flow. Eddy formations about areas of obstructions (glottic narrowing, branching of bronchi) disturbing laminar flow are responsible for this. Classic methods of measuring airway resistance, estimating both flow and alveolar pressure, can ignore this nonlinearity except for noting the flow value at which the airway resistance is measured, but other methods of measuring airway resistance without estimating alveolar pressure must take it into consideration.

responding breast milk Ca/P ratio in 6-day old infants suggests that the relationship is not critical for breast fed infants Available evidence for infants fed cows milk preparations of low mineral content support this concept (2) However it must be recognised that human milk is of inconstant composition and that the infants studied in this paper may have received varying mineral loads during their first 6 days of life

The variability of the data reported indicate the difficulties in determining the optimal mineral content of infant milk formulae This has become increasingly important in view of attempts to produce milk more closely resembling human milk in composition and in the recent attempts to establish internationally accepted standards for infant foods (12) The data suggest that human infants can tolerate a wider range of Ca/P ratios at low mineral loads than has hitherto been supposed although this does not take account of other factors such as the amount and nature of the milk fat that might influence absorption or utilisation of calcium

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AN ADDITIVE METHOD FOR AIRWAY RESISTANCE MEASUREMENT

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ABSTRACT Kureš, H. (Physiological Laboratory of the Paediatric Clinic, Karolinska Hospital, Stockholm, Sweden and the Research Institute for Child Development, Charles University Prague, Czechoslovakia). An additive method for airway resistance measurement. Acta Paediatr Scand, 63: 351 1974.—In order to increase the possibilities of determining the airway resistance especially in children, an additive method has been developed which does not require cooperation of the subject being examined nor estimation of alveolar pressure. By means of mathematical derivations it is shown that the ratio mouth pressure difference and flow difference at instant change of additive resistances gives the airway resistance in laminar as well as nonlaminar flow. Besides a recording unit, the equipment consists of a special pick-up tube with three parts: a flow pick-up, a mouth pressure pick-up and two added resistances with means for alternating between them. The method has been tested by using artificial flow resistances and by means of a conventional body plethysmographic method in 4 healthy adults, 10 healthy children and 8 asthmatic children. A correspondence within ±4% of obtained values was found with no significant differences. The method does not call for any artificial breathing manoeuvre, does not cause any unpleasant sensation and allows repetition at short intervals.

KEY WORDS: Airway resistance, children

Classic methods of airway resistance measurement, i.e. the body plethysmographic method and the method of air flow interruption still suffer from some disadvantages in routine use. The former needs the cooperation of the subject being examined and the necessary equipment is expensive while the latter does not seem to yield correct results. Attempts have therefore been made to develop another method which would eliminate these disadvantages and would not require alveolar pressure estimation, which is the cause of most troubles. One such attempt is the additive method the theory apparatus and use of which are presented in this paper

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The basic relation from which the airway resistance is derived is:

$$P_{alv} = R_{aw} \dot{V} \qquad (1)$$

where P_{alv} = alveolar pressure as a pressure difference between alveoli and ambient atmosphere, representing a driving pressure across the airway resistance, R_{aw} = the airway resistance $\dot{V}$ = volume rate of change

The airway resistance is then expressed

$$R_{aw} = P_{alv} / \dot{V} \qquad (2)$$

The relation between alveolar pressure and flow in human airways was found to be nonlinear. Therefore the airway resistance value of a bronchial tree is not constant but changes with flow. Eddy formations about areas of obstructions (glottic narrowing, branching of broncial) disturbing laminar flow are responsible for this. Classic methods of measuring airway resistance estimating both flow and alveolar pressure can ignore this nonlinearity except for noting the flow value at which the airway resistance is measured, but other methods of measuring airway resistance without estimating alveolar pressure must take it into consideration.

The nonlinear relation between alveolar pressure and flow during nonlaminar flow (eddy flow) can be expressed in several ways but most commonly by Rohrer's equation (1):

$$P_{alv}=K_1\dot{V}+K_2\dot{V}^2 \quad (3)$$

where the nonlinearity is characterized by coefficients K_1 and K_2 combined respectively with the first and second power of flow

Ainsworth's equation (2) yields an almost identical description for a wide range of flow values:

$$P_{alv}=K_{aw}\dot{V}^x \quad (4)$$

where the nonlinearity is characterized by the power of flow (in case of air flow $1<x<2$).

The latter equation (4) conforms to the basic equation (1) Both these equations represent a parabolic function if the power of flow in equation (1) is considered to be different from 1 Then also the airway resistance (R_{aw}) and coefficient of airway resistance (K_{aw}) are comparable and as this is convenient for the following derivation Ainsworth's equation will be used hereafter

Theory

The theory of the additive method of airway resistance measurement will be propounded firstly for an ideal case of stabilized laminar flow and its validity for eddy flow will be proved later

When an additional resistance is connected in series with a flow resistance flow will decrease assuming equal driving pressure When two different additional resistances are alternately connected in series with a measured airway resistance then two alternative situations with different flow values obtain. Both these situations can be described by two similar equations conforming to the basic equation (1):

$$P_{alv}=(R_{aw}+R_1)\dot{V}_1 \quad (5)$$
$$P_{alv}=(R_{aw}+R_2)\dot{V}_2$$

where R_{aw}=common measured airway resistance R_1 R_2= the added resistances $\dot{V}_1$ $\dot{V}_2$=the flow value in each respective situation

Assuming an equal alveolar pressure in both situations the two previous equations can be combined as follows

$$(R_{aw}+R_1)\dot{V}_1=(R_{aw}+R_2)\dot{V}_2 \quad (6)$$

The identity of alveolar pressure in both situations occurs at the instant of exchange of both added resistances and will be discussed later

Since the value of alveolar pressure is eliminated in equation (6) the airway resistance can theoretically be calculated using only the values of the two added resistances and their corresponding flow values:

$$R_{aw}=(R_2\dot{V}_2-R_1\dot{V}_1)/(\dot{V}_1-\dot{V}_2) \quad (7)$$

Formula (7) could be used only for stabilized laminar flow but not for eddy or turbulent flow For the nonlinear relation between alveolar pressure and flow the added resistances have to be constructed with nonlinear characteristics (see below). In such case the added resistances could not be defined unambiguously by simple multiples $R_1\dot{V}_1$ and $R_2\dot{V}_2$ for eddy or turbulent flow

But $R_1\dot{V}_1$ and $R_2\dot{V}_2$ in fact represent pressure differences P_1 and P_2 across the added resistances R_1 and R_2 (accordingly to equation (1)) and equation (7) can be re-written in a more convenient form as follows

$$R_{aw}=(P_2-P_1)/(\dot{V}_1-\dot{V}_2) \quad (8)$$

Pressure differences P_1 and P_2 can be easily measured as mouth pressure values at the point where the added resistances join the measured resistance. By estimating the pressure difference across the added resistances corresponding to a given flow value the added resistances become perfectly defined even when constructed with non-linear characteristics.

Although equation (8) was derived for an assumed stabilized laminar flow it can in addition be shown to be valid for other types of flow (eddy and turbulent flow).

When previous derivation is based on equation (4) instead of equation (1) the following equation analogous to equation (7) is obtained.

$$K_{aw}=(K_{R_2}\dot{V}_2^x-K_{R_1}\dot{V}_1^x)/(\dot{V}_1^x-\dot{V}_2^x) \quad (9)$$

where K_{aw} K_{R_1} K_{R_2}=coefficients of airway resistance and respectively the two added resistances

Equation (9) can be adapted for airway resistance calculation for conditions of non-laminar flow in the following way the interrelation between the coefficient of airway resistance (K_{aw}) and the airway resistance (R_{aw}) is derived by combining equations (1) and (4):

$$R_{aw}\dot{V}=K_{aw}\dot{V}^x \text{ and therefore} \quad (10)$$
$$K_{aw}=R_{aw}\dot{V}/\dot{V}^x$$

The adaptation of equation (10) for the additive method where two situations with two flow values alternate, is the following:

$$K_{aw}=R_{aw}(\dot{V}_1-\dot{V}_2)/(\dot{V}_1^x-\dot{V}_2^x) \quad (11)$$

Replacing K_{aw} in equation (9) by the right side of equation (11) and solving the new equation for R_{aw} the following equation is obtained:

$$R_{aw}=(K_{R_2}\dot{V}_2^x-K_{R_1}\dot{V}_1^x)/(\dot{V}_1-\dot{V}_2) \quad (12)$$

This conforms with formula (7), being identical when $x=1$ but valid for any type of flow According to equation (4) here too $K_{R_1}\dot{V}_1^x$ and $K_{R_2}\dot{V}_2^x$ can be replaced by corresponding pressure differences across the added resistances, i.e. by mouth pressure values P_1 and P_2 in the two situations Then formula (8) is again obtained.

Evidence has been given to prove that the additive method of estimating airway resistance and the final formula (8) can be considered correct also in nonlaminar flow if an important condition is fulfilled: all resistances in the system (the airway resistance and

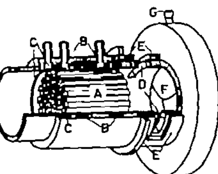


Fig. 1. The pick-up tube in partial section. For detailed description, see text.

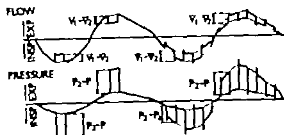


Fig. 2. A schematic drawing of flow and mouth pressure curves during alternation of the added resistances.

the added resistances) should have equal flow-pressure characteristics, i.e. the relation between the flow and the pressure difference across each of them should be equal.

Apparatus

The equipment required for measuring airway resistance by the additive method consists of a special pick-up tube and a recording unit.

The pick-up tube contains three parts, a flow pick-up, a mouth pressure pick-up and the two added resistances with means for alternating between them. A design of the pick-up tube which has proved most convenient is illustrated schematically in Fig. 1.

The flow signal is recorded with aid of a Fleisch-type pneumotachograph. An auxiliary flow resistance with linear flow-pressure characteristics, manufactured from a corrugated metal foil (*A*), is inserted in the tube. Two pressure signals across this resistance are led through lateral slits in the wall of the tube via two collecting chambers (*B*) to a differential electromanometer.

The mouth pressure signal is led through a third slit via collecting chamber (*C*) to a second electromanometer.

The switching in and out of the additional resistance is achieved by means of shutter (*F*) which alternately opens and closes the main orifice of the pick-up tube. The shutter is operated either manually or electromagnetically by a Bowden cable connected at (*G*).

When the shutter is open the air flows axially and the smaller resistance, represented by the resistance of the corrugated insertion, is in the circuit. When the shutter is closed, the air stream is deflected through lateral slits (*D*) which form the greater added resistance. The orifice of these slits, and consequently the magnitude of the added resistance can be regulated by turning ring (*E*) to close them partially.

The dead space of the pick-up tube is less than 18 ml. The flow is reproduced linearly up to about 600 ml/sec.

The simplest recording unit consists of two pressure transducers, one differential for recording flow and one plain for recording mouth pressure, combined with a two-channel recording apparatus. It is advantageous, but not absolutely necessary, to add a third channel for simultaneously recording the spirogram obtained by electronically integrating the flow signal.

Calibration of flow and pressure is performed with aid, respectively, of a rotameter and water manometer.

Measuring procedure

The subject breathes through the pick-up tube provided with a mouth piece while the shutter is alternately opened and closed about four times a second and the flow and mouth pressure signals recorded simultaneously. Both curves are similar in shape (Figs. 2 and 3). Alternation of the resistances causes a step-like deformation in each curve, but in opposite senses. When the shutter is opened the flow rises and the mouth pressure decreases and vice versa.

The necessary values for airway resistance calculation are read at the instants of resistance change. The height of the coinciding steps corresponds to the values necessary for formula (8): flow difference $V - V$ and mouth pressure difference $P - P$. The details concerning the reading are discussed later on.

The airway resistance can therefore be evaluated several times during one breathing cycle by converting the measured deflections in millimeters to litres per second and, respectively, centimetres of water.

Verification

The accuracy of the additive method has been verified by tests with model resistances and human subjects.

Three different artificial flow resistances were manufactured of porous plastic with flow-pressure characteristics similar to those of human airways. Values were estimated according to formula (2) with the aid of a Pitot tube: 1.38, 4.42 and 7.50 cm H_2O/l/sec at flow 0.5 l/sec. Measurements were performed at different flow rates: 0.1, 0.3 and 0.5 l/sec. Comparing these values with similar ones obtained by means of the additive method, correspondence was found to be better than +4.2% and −3.8%.

Airway resistances measured with the additive method and with the conventional body plethysmographic method were compared in 22 subjects, 4 healthy adults, 10

Elema Mingograph 81 with two EMT 31 electromanometers, pressure transducers EMT 32 and EMT 33 and integrator EMT 41. Siemens Ab U.B. med., Erlangen, BRD

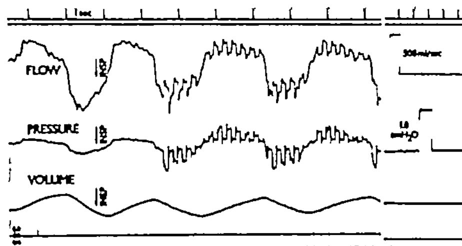


Fig 3 An original record of flow and mouth pressure curves before and during alternation of the added resistances. The volume curve was obtained by electronic integration of the flow signal.

healthy children and 8 asthmatic children with mild dyspnea. Measurements were performed successively in an identical body position (sitting in the body box) with mouth breathing. Average airway resistances of 15 breaths in each subject were compared and calculated as a mean value between inspiratory and expiratory resistance at maximal flow during normal breathing. Neither group of results was found to be statistically different at the 0.05 level. Individual values for each type of measurement are plotted against each other in Fig. 4

DISCUSSION

Several attempts have been made to design a method of estimating airway resistance using the additive principle

Dirnagl s method (3) based on added resistances with linear flow pressure characteristics in a special pick up tube (4 5) has been used by several workers The airway resistance is here calculated from the pressure changes and the constant values of the two resistances only

Sobol s method (6) also uses for evaluation only the constant values for the added resistances and flow changes

Neither of these methods takes into account the influence of non-laminar flow and both use different formulae from those reported here The theory presented above shows the resulting consequences on the accuracy of measurement.

The assumption of equal alveolar pressure in both situations with different added resistances needs to show that the step-like changes of mouth pressure are not reflected in similar changes of alveolar pressure during measurement Therefore the following experiment was realized The "to and fro" air flow was led through an artificial airway resistance and through the device for its measurement being generated with aid of a compliant rubber bellows Both the flow and mouth pressure signals were recorded simultaneously with the pressure inside the bellows (corresponding with the alveolar pressure in the lung) No changes in bellows pressure corresponding to the added resistances exchange were noticed (Fig. 5)

Even the added resistances exchange itself does not influence the alveolar pressure physically though the sum of the original

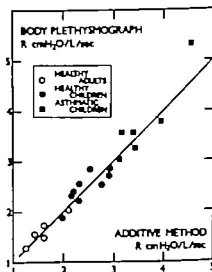


Fig 4 A comparison of airway resistance values obtained by the additive method and by the body-plethysmographic method

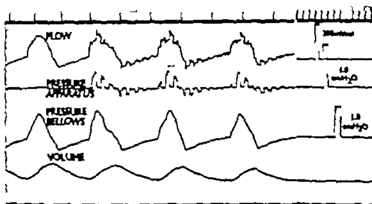


Fig 5 An original record of flow apparatus pressure and bellows pressure signals obtained in a model experiment. The volume curve was obtained by electronic integration of the flow signal.

and the added resistance represents a higher value during measurement. It may cause some reflex changes of the driving force and thus of the alveolar pressure too But such changes of alveolar pressure such as its cyclic inspiratory and expiratory oscillation are much slower than the very short changeover time of added resistances. Hence the assumption of equal alveolar pressure in both situations with both added resistances can be considered correct.

Accuracy of measurement is ensured if the pick-up tube is designed according to the theory The flow-pressure characteristics of human airways have been estimated by the following coefficients in Rohrer's form of equation (7):

$P_{aw} = 1.2\ V + 0.3\ V^2$

The accordance of resistive properties of greater additional resistance with that of air way resistance is obtained by specially-shaped lateral slits (Fig. 1D) in the described device.

If the influence of non-laminar flow is neglected, the total error may approach ±30% even in the case of normal patients, the absolute value of the error being dependent on the flow

Concerning the resistive property of the smaller added resistance its flow-pressure characteristics must be kept linear to get an adequate flow record Any divergence from theory can be neglected here because the resistance of the open pick-up tube is so small (less than 0.5 *cm* H_2O/*l/sec*)

The accuracy of measurement poses several pre-requisites.

The steps in both curves should be high enough to enable precise reading to be made It concerns principally the flow steps Optimal results have been obtained when both the airway resistance and the greater added resistance were equal. Then the height of flow steps represented about a *half of the* pressure steps and the sensation for the patient was not disagreeable

If the airway resistance is too *great in com*parison with the added resistance the flow steps can become indistinct. This can happen in patients suffering from asthmatic dyspnea or in small children In such cases it is useful to make the added resistance greater by a partial closing of the lateral resistive slits with aid of the turnable ring.

The changeover time from one added resistance to the other should be kept to a minimum to ensure clearly-marked inflexion points at the top and bottom of each step This enables precise readings to be made suppresses the influence of inspiratory and expiratory flow oscillations and precludes the effect of possible reflex reactions on the changed resistance. Most convenient for this purpose is a centrally-closing shutter

However sudden increases and decreases of the resistance cause an instantaneous com-

pression and decompression of the air column inside the airways This is evidenced by a spike shaped deformation in both curves at the instants of added resistance change (Figs 2 and 3)

The height of these spikes has been found both in flow and pressure record to be proportional to the mean height of the steps Thus this deformation need not be considered disadvantageous on the contrary it enables sharper reading to be made from bottom to the top of spikes In this case the spikes should not be suppressed by damping the recording systems since uneven damping could introduce more inaccuracy than phenomenon itself

Nevertheless the phenomenon might theoretically cause a slight difference between the airway resistance values estimated at opposite switch points i e when the added resistance is switched to its maximum value and vice versa. The switching directed from greater to smaller added resistance has been found to be most convenient for evaluation

The airway resistance can be estimated several times during one breathing cycle depending on the frequency of exchange The manually-operated shutter can be comfortably closed about four times a second So 12–15 airway resistance values can be read in different stages of the breathing cycle It facilitates dynamic follow-up of resistance changes dependent on flow rate or volume and separate evaluation of inspiratory and expiratory resistance to be made Simultaneous registration of a spirogram obtained by integrating the flow signal is useful in this case Verification of the additive method has shown that results are correct and comparable with those obtained by means of the *body plethysmographic method*

The use of the device described and the method are simple enough to allow repetition at short intervals It is useful when performing inhalation tests in asthmatic subjects. An examination does not call for any artificial breathing manoeuvre and does not cause any unpleasant sensation Therefore it can be used even in dyspneic patients and in children

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RENAL INVOLVEMENT IN SCHÖNLEIN HENOCH PURPURA

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ABSTRACT Koskimies, O., Rapola, J., Savilahti, E. and Vilska, J (The Children s Hospital, University of Helsinki, Helsinki, Finland). Renal involvement in Schönlein-Henoch Purpura. Acta Paediatr Scand, 63: 357 1974.—During the period 1964–71 Schönlein-Henoch syndrome was found in 91 children. Fifty-four showed no signs of renal disease and recovered rapidly Eighteen patients with haematuria or proteinuria lasting less than 4 weeks also made full recovery Nineteen patients had persisting urinary abnormalities and were followed-up clinically and with biopsies. Immunofluorescent and electron microscopic studies were performed in 6 patients.

Histopathological changes correlated well with the severity of the disease. Generalized proliferative glomerulonephritis, sometimes with glomerular fibro-epithelial crescents, signified severe disease with protracted course. Most of these cases had heavy proteinuria and often nephrotic syndrome. The outcome after a follow-up of 3 years was good, however Twelve patients recovered completely and 6 were clinically symptomless but showed minor urinary abnormalities. Only one died and she had showed fulminant clinical and histopathological glomerulonephritis from the beginning of the disease.

Immunofluorescent studies showed positive reactions in the mesangium and glomerular capillaries in 4 patients. In the electron microscopy dense deposits were found at the corresponding sites. In two late biopsies immunofluorescence was negative despite definitive histological changes suggesting disappearance of the immunopathogenetic effectors of the nephritis.

KEY WORDS: Nephrotic syndrome, glomerulonephritis, hypersensitivity immunologic disease, vascular purpura

The Schönlein-Henoch (SH) syndrome pur puric rash alimentary and joint involvement usually with fever and oedema, is a well-known entity The incidence of renal involve ment in this syndrome varies from appr 20 to 90% in different reports (1 11) Kidney disease is of utmost importance since glomerulonephritis is the major cause of serious disease and death among patients with SH-syndrome

We report here the correlation between the clinical course and the light microscopy elec tronmicroscopy and immunofluorescent findings of the patients with SH-nephritis. These data are also correlated with the long term prognosis of the patients The analysis of 91 patients with SH-syndrome also gives a rough estimate of the incidence of renal involvement in this disease in Finnish children. All patients with SH-syndrome admitted to the Children s Hospital University of Helsinki in 1964–1971 were studied

PATIENTS AND METHODS

The criteria for inclusion in the investigation were pur puric rash characteristically affecting the limbs and buttocks and alimentary or joint involvement (or both) Other diseases with similar manifestations were ex cluded. A history of infection occuring in the 4 weeks preceding the syndrome was present in 80% of the children and evidence of streptococcal etiology (positive culture elevated ASO titre) was recorded in 14 out of 91 patients. Children with pre-existing kidney disease in the history were not included in the study All patients

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Table 1 *Clinico-pathological correlations and outcome of 19 patients with Schönlein Henoch nephritis*

Pa-tient no	Age years/ Sex	Histology grade			Main renal presentations					
		1	2.	3 Biopsy	Hemat uria	Protein uria	Heavy protein-uria	Nephritic syndr	Nephrotic syndr	Renal insuf ficiency
1	5 M	I			+					
2	4 M	I			+	+				
3	7 F	I			+	+				
4	6 M	II			+					
5	3 M	II			+	+				
6	10 M	II	II		+	+				
7	1? F	II			+	+				
8	12 M	II			+	+				
9	14 M	II			+	+				
10	9 M	II	I	I	+	+		+		
11	6 M	III			+	+				
12	10 M	III			+	+				
13	11 F	III			+	+				
14	1? M	III	III		+	+				
15	7 M	III			+		+			
16	10 F	III			+		+		+	
17	1? F	III	I	I	+		+		+	
18	11 M	IV	II		+		+		+	
19	13 F	V	V	V	+		+	+	+	+

Followed-up less than ? years First renal biopsy was performed within 3 months of the onset of the disease except on patients 7 11 1? and 13 These biopsies were performed 13 6 60 and 11 months from the onset respectively Rec =recovered Impr =improved.

were followed for at least 1 year after the disease and those with renal involvement for at least ? years

The urine analysis consisted of a reagent strip test for blood and protein microscopy of unspun specimens in a counting chamber and examination of the centri fuged deposit Colony count or dip slide (Uricult®) culture was also performed Urinary protein was measured in most cases from 1? hour overnight collections of urine or alternatively the determination was made on the first voided morning specimen. Protein-free urine proteinuria and heavy proteinuria were defined as protein excretion less than 4 mg more than 4 mg or more than 40 mg per hour per m² of body surface area (BSA) respectively The urine analysis was performed 3 to 7 times a week during the first month, whereafter analyses were performed less frequently according to the decision of the physician in charge of the patient.

The biopsy specimens were obtained by percutaneous technique All 27 specimens from 19 patients were studied by light microscopy and in addition 6 biopsies from 6 patients were studied by electron microscopy and 7 biopsies from 6 patients by immunofluorescent technique For light microscopy the specimen was fixed in 10% neutral buffered formalin and since 1968 in alcoholic Bouin s solution After fixation the specimen was dehydrated embedded in paraffin and appr 2–3 μm thick sections were stained with hematoxylin and eosin periodic-acid-Schiff (PAS) periodic-acid-silver methenamin (PASM) and Masson s trichrome method All histological specimens were studied by one of us (J R)

The specimen for electron microscopy was fixed in 2% glutaraldehyde postosmicated and embedded in Epon 81? Ultrathin sections were double-stained with uranyl acetate and lead citrate The electron microscope employed was the Zeiss EM 9 A

Fresh-frozen samples from biopsies were cut in 4 μm sections in a cryostat microtome at −70°C and processed for direct immunofluorescent study as described earlier (9) Commercial goat fluorescein isothiocyanate (FITC) conjugated antisera to human IgG IgM IgA and fibrin were purchased from Hyland Laboratories (Los Angeles Calif) Rabbit anti-β-1C and sheep anti IgE sera were conjugated with FITC (7) The monospecificity of the antisera, their lack of cross-reactivity and F/P ratios were checked as reported elsewhere (9 10). The F/P ratio of the antifibrin antiserum was 1 4

RESULTS

Altogether 91 patients with SH syndrome fulfilling the criteria for this study were investi gated (Fig 1) No hemat or proteinuria or other signs of renal disease were observed in 54 patients during the period of 4 weeks from the onset of the disease All these patients recovered completely and only 1 late-occur rence transient hematuria was observed

Duration of abnormal urinary finding (months)	Outcome >2 years
4	Rec.
1	Rec.
2	Rec.
1	Rec
18	Rec.
>32	Impr
>15	Impr
1	Rec
>68	Impr
2	Rec.
20	Rec.
>72	Impr
23	Rec.
17	Rec.
> 4	Impr
> 6	Impr
32	Rec.
15	Rec.
10	Death

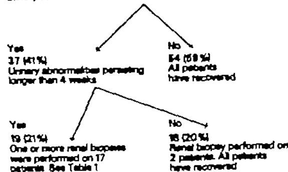


Fig 1 The distribution of 91 patients with Schönlein-Henoch syndrome in various subgroups according to signs of renal involvement.

Urinary abnormalities were recorded in 37 patients. Of these the urinary findings of 18 were normalized within 4 weeks. A renal biopsy was performed on two of these patients and normal histology or minor changes were observed (Table 1 patients 2 4) Signs of recurrences of the nephropathy were not observed in outpatient check-ups

Clinico-pathological correlations and outcomes

Nineteen patients had urinary pathology lasting more than 4 weeks. A total of 25 kidney biopsies were performed on 17 of these patients. The clinico-pathological data and outcome of the patients are presented in Table 1 The histological picture varied from normal to severe glomerulonephritis. The most common lesion was focal and local mesangial hypercellularity accompanied by an increase of PAS- and PASM-positive mesangial matrix The basic focal and local nature of the affection was maintained even in the specimens showing more generalized lesions variation in extent of lesions between different glomeruli and individual segments within single glomeruli was evident The severity of the histopathological lesions was classified in five grades grades I–II are considered as slight and III–V as severe histopathological changes (for details see Meadow et al (8))

The renal presentation ranged from microscopic hematuria to rapidly progressing glomerulonephritis and renal insufficiency The most common observation however was a combined hematuria-proteinuria Nine patients with hematuria-proteinuria or isolated hematuria belonged to histopathological groups I–II and four to group III One patient with grade II histology had an acute nephritic syndrome (hematuria with elevated blood urea nitrogen and oliguria) of short duration in the beginning of the disease All of these 14 patients have recovered (no clinical or laboratory signs of disease) or improved (clinically healthy but microscopic hematuria and/or residual proteinuria 4–10 mg/h/m² of BSA) (Table 1)

Four children out of 5 with hematuria and massive proteinuria developed nephrotic syndrome (serum albumin less than 2.5 g/100 ml and urinary protein excretion more than 40 mg/h/m² of BSA) The disease of one of these patients (No 19) initially resembled acute nephritis which later developed into nephrotic syndrome Gross destruction and epithelial crescents in almost all glomeruli were already

Table 1 *Clinico-pathological correlations and outcome of 19 patients with Schonlein-Hen nephritis*

Pa tient no	Age years/ Sex	Histology grade			Main renal presentations					
		I	2.	3 Biopsy	Hemat uria	Protein uria	Heavy protein-uria	Nephritic syndr	Nephrotic syndr	Renal insuf ficiency
1	5 M	I			+					
2	4 M	I			+	+				
3	7 F	I			+	+				
4	6 M	II			+					
5	3 M	II			+	+				
6	10 M	II	II		+	+				
7	1? F	II			+	+				
8	1? M	II			+	+				
9	14 M	II			+	+				
10	9 M	II	I	I	+	+		+		
11	6 M	III			+	+				
12	10 M	III			+	+				
13	11 F	III			+	+				
14	12 M	III	III		+	+				
15	7 M	III			+		+			
16	10 F	III			+		+		+	
17	1? F	III	I	I	+		+		+	
18	11 M	IV	II		+		+		+	
19	13 F	V	V	V	+		+	+	+	+

Followed-up less than ? years First renal biopsy was performed within 3 months of the onset of the disease except on patients 7 11 12 and 13 These biopsies were performed 13 6 60 and 11 months from the onset re spectively Rec =recovered Impr =improved

were followed for at least 1 year after the disease and those with renal involvement for at least ? years

The urine analysis consisted of a reagent strip test for blood and protein microscopy of unspun specimens in a counting chamber and examination of the centri fuged deposit Colony count or dip slide (Uricult®) cul ture was also performed Urinary protein was measured in most cases from 1?-hour overnight collections of urine or alternatively the determination was made on the first voided morning specimen. Protein-free urine pro teinuria and heavy proteinuria were defined as protein excretion less than 4 mg, more than 4 mg or more than 40 mg per hour per m² of body surface area (BSA) re spectively The urine analysis was performed 3 to 7 times a week during the first month whereafter analyses were performed less frequently according to the decision of the physician in charge of the patient

The biopsy specimens were obtained by percutaneous technique All 27 specimens from 19 patients were stu died by light microscopy and in addition 6 biopsies from 6 patients were studied by electron microscopy and 7 biopsies from 6 patients by immunofluorescent tech nique For light microscopy the specimen was fixed in 10% neutral buffered formalin and since 1968 in alco holic Bouin s solution After fixation the specimen was dehydrated embedded in paraffin and appr ?-3 μm thick sections were stained with hematoxylin and eosin periodic-acid-Schiff (PAS) periodic-acid-silver methe namin (PASM) and Masson s trichrome method All histological specimens were studied by one of us (J R)

The specimen for electron microscopy was fixed in ?% glutaraldehyde postosmicated and embedded in Epon 81? Ultrathin sections were double-stained with uranyl acetate and lead citrate The electron microscope em ployed was the Zeiss EM 9 A

Fresh-frozen samples from biopsies were cut in 4 μm sections in a cryostat microtome at −?0°C and processed for direct immunofluorescent study as de scribed earlier (9) Commercial goat fluorescein isothio cyanate (FITC) conjugated antisera to human IgG IgM IgA and fibrin were purchased from Hyland Laboratories (Los Angeles Calif) Rabbit anti-β-1C and sheep anti IgE sera were conjugated with FITC (7) The monospeci ficity of the antisera their lack of cross-reactivity and F/P ratios were checked as reported elsewhere (9 10). The F/P ratio of the antifibrin antiserum was 1 4

RESULTS

Altogether 91 patients with SH syndrome ful filling the criteria for this study were investi gated (Fig 1) No hemat or proteinuria or other signs of renal disease were observed in 54 patients during the period of 4 weeks from the onset of the disease All these patients recovered completely and only 1 late-occur rence transient hematuria was observed



Fig. 3. Electron micrograph of a part of a glomerular segment. Mesangial hypercellularity and increased matrix is evident. Basement membrane of the capillaries is unevenly thickened. Small intramembranous electron-dense deposits (*D*) are seen at the junction of capillary and mesangial basement membrane. *M* = mesangium. *C* = capillary lumen. ×6 000

cent-positive biopsies stained with anti-β-1C and four with anti-Ig-sera. Staining patterns were roughly similar in all positive antisera. One primary biopsy and one follow-up biopsy were negative while in one follow-up biopsy with a more severe histological picture positive staining was seen.

The six biopsies studied by electron microscopy were histologically graded from I to III. All except one were from initial biopsies. No follow-up biopsies from the same patient were available. The range of severity of the lesions as judged by light microscopy was not evident in the electron microscopy possibly due to the focal nature of the glomerular involvement and the limited sample available for the electron microscopy.

Increased mesangial basement membrane like matrix, swelling of the endothelial cytoplasm, protrusion of the mesangial cytoplasm into the capillary lumen, focal thickening and scalloping of the endothelial aspect of the basement membrane were found in every biopsy. Sparse and small-size electron-dense deposits located at the endothelial aspect or within the basement membrane were usually

Table 2 *Immunofluorescent study of biopsy specimens*

Patient no	Number of biopsy	Anti-β-IC	Anti-IgG	Anti-IgM	Anti IgA	Anti IgE	Anti-fibrin	Staining patterns
6	1	+	-	+	-	NS	NS	Gen. Seg. Gr Cap Mes Other vessels also stained
	2	-	-	-	-	-	-	-
7	1	+	+	-	+	-	+	Gen Seg. Gr Mes Cap
8	1	-	-	-	-	NS	-	-
11	1	+	+	-	-	NS	+	Gen. Seg. Gr Mes Cap
14	2	+	+	-	+	NS	NS	Gen Seg. Gr Mes Cap
15	1	+	+	-	+	NS	+	Gen. Seg. Gr Mes Cap.

Gen. =general, affecting all glomeruli in the specimen
Seg. =segmental in glomeruli
Gr =Granular
Mes. =staining in the mesangial area
Cap =staining along capillary walls.
NS =not studied.



Fig 2 Biopsy specimen stained with anti-β-IC antiserum (patient no 15) Coarsely granular and thick fluorescence is seen mainly in the mesangial area but also along capillary walls Fluorescence is also seen in the wall of a blood vessel at the top of the figure Original magnification ×400

to be seen in the first biopsy 2 months after the onset of disease (histological grade V) She died 10 months after the onset of disease in renal failure The other 4 patients also had severe histological changes which were classified in grades III–IV The outcome in 3 of these children was eventually excellent but one has so far been followed only 6 months.

No systematic study of the effect of treatment with corticosteroids and cytotoxic drugs was possible because a number of patients 1964–69 were treated with ACTH and corticosteroids and some children 1970–72 with cyclophosphamide

Immunofluorescent and electron microscopy studies

In four out of five initial biopsies positive fluorescence was observed (Table 2) Staining was mostly coarsely granular and present predominantly in the mesangial area in all but one case although it was frequently extended to capillary walls (Fig 2) All immunofluores

(12 4). No immunocomplexes were seen in a follow-up biopsy (case 6) nor in one initial biopsy (case 8) made 2 months after the commencement of the disease both specimens showed grade II histopathological changes. This suggests that the disappearance of immunocomplexes precede complete histopathological healing.

The presence of immunoglobulins and complement in granular fashion combined with the deposits detected by electron microscopy suggest that the glomerular injury in SH nephritis is mediated by immunocomplexes as in several other types of human and experimental glomerulonephritis.

ACKNOWLEDGEMENT

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found (Fig 3) They were sometimes also seen in the mesangial matrix

In one specimen obtained 18 months after the initial biopsy but still graded histologically as III no deposits were found

In one case layering of fibrin in the capillary lumen and in another in the Bowman s space were seen Polymorphonuclear cells were seen in the Bowman s space in one case but never in the mesangium nor in the capillary loops Collagen deposits were not encounte red

DISCUSSION

The frequency of renal involvement in SH syndrome varies in different published series (1 11) This variation may be due to the se lection of patients included in the study and/ or local variability of the largely unknown hypersensitizing antigens and most important the criteria for assessing the renal involve ment For instance pathological changes have been reported in biopsies of some patients who did not show urinary abnormalities or other signs of kidney disease (3) We based our initial criterion of the renal injury on the urinary findings 41% of the patients showed hematuria as the minimum sign of the kidney disease Most of our patients were first re ferrals from the practitioners and out patient clinics of the hospital district but some were referred from other hospitals because of the kidney disease This selection in creases somewhat the incidence of the renal involvement in our series in relation to an un selected HS population

The retrospective evaluation of the material showed that the existence and the duration of the urinary abnormalities had a clear prog- nostic value All 54 (59%) patients without pathological urinary findings had excellent prognosis The rest of the patients were divided into two groups those whose urinary findings persisted over 4 weeks (19 patients 21%) and those whose urine cleared in less than 4 weeks (18 patients 20%) (Fig 1) Only children with persisting urinary abnormalities were in potential danger All other patients recovered completely notwithstanding re- lapses of purpura episodes

Heavy proteinuria (over 1 g/24 hours) nephritic and nephrotic syndrome either alone or in combination are considered as markers of potentially unfavourable prognosis (4 8) The presence of these features in our series was usually followed by a severe and pro- tracted clinical course of the disease and grade III–V changes in biopsy However 4 out of 5 of these patients eventually recovered or clearly improved none of them developed slowly progressive renal disease The pres- ence of epithelial crescents of the Bowman s capsule in SH nephritis have proved to be a most important prognostic marker (4 8) Only one patient in our series had a fulminant form of renal disease with crescents in almost all glomeruli in the biopsy specimen Her disease had a fatal outcome

Similarly favourable prognosis of children with SH nephritis has also been observed by others (6 8) this differs markedly from the prognosis of adult patients with this disease Mortality of appr 30% has been reported in adults with SH nephritis (2)

The histological lesion in SH nephritis is variable and there is no single pathognomonic lesion The most common finding however is focal proliferative glomerulonephritis (13 5 4) Our findings are in agreement with these studies and corroborate the observations of Meadow et al (8) that focal and local varia- tion of the lesions is detectable even in cases which have been classified as diffuse glom- erulonephritis In positive cases studied by immunofluorescent technique all glomeruli in the sections were affected regardless of the focal lesions seen in histology confirming the findings of Habib & Levy (4) The intensity of the staining in single glomeruli however showed a segmental pattern Staining confined mostly to the mesangial area and the exten- sion to capillary walls is similarly found in earlier reports and this agrees with electron microscope findings in this and earlier studies

Table 1 *Clinical data on control subjects and patients with hypopituitarism or Silver Russell syndrome*

Subjects	Sex	CA (yrs)	HA (yrs)	BA (yrs)	Deficiency ACTH	Deficiency TSH	Deficiency HGH	Treatment
Control								
B B.	M	3 5/12	3 9/12	?	–	–	–	None
B. E	M	6 9/12	6 0/12	?	–	–	–	None
R. B	M	6 10/12	6 3/12	?	–	–	–	None
Hypopituitary								
C. D	M	7 8/12	4 6/12	5 6/12	–	±	+	None
P D	M	13	9	11	–	+	+	Thyroid extract
M. V	F	13	6 9/12	8	–	–	+	None
R B.	M	14 6/1	8	7	–	+	+	Thyroid extract
H B.	M	18	8 3/12	15	+	+	+	Thyroid extract, cortisone
A N	M	8	4 6/12	4	–	+	+	Thyroid extract, HGH
E. G	M	9 6/12	6	6 6/12	–	+	+	Thyroid extract HGH
R. R	F	17	8 9/12	10	–	+	+	Thyroid extract, HGH
Silver-Russell								
K. S	M	5 9/12	3 6/12	3 6/12	–	–	–	HGH
V M	M	11 6/12	8 3/12	10	–	–	–	HGH

subjects of another report (5). Bone age was determined by comparison with the standards of Greulich & Pyle (3) Anthropometric measurements were evaluated with the growth tables of Stuart & Meredith (18) GH deficiency was documented (Table 2) by the absence of growth hormone release following the administration of insulin (0.1 U/kg body weight intravenously) arginine (0 5 g/kg intravenously for 30 minutes) and glucagon (0.1 mg/kg intramuscularly). The tests were performed when the subjects were euthyroid for at least 6 months When necessary appropriate amounts of thyroid extract had been administered One patient needed also cortisone replacement therapy which was discontinued 48 hours before the study The 2 children with Silver-Russell syndrome had no known endocrine disorders Detailed data on these latter patients were published previously (1). Three out of the 8 GH-deficient patients and the 2 Silver-Russell patients had been treated since 2 years with human GH (HGH) prepared by the Organon Co. (Oss, the Netherlands) according to the extraction procedure of Raben (17) They received two doses weekly of 5 mg intramuscularly Although in the 2 Silver-Russell patient pituitary GH release was not defective exogenous HGH was given following a preliminary report of Tanner & Ham (19)

METHODS

The effect of c AMP on GH secretion was studied in the previously mentioned subjects after having obtained informed and written consent from their parents Dibutyryl c AMP was used as it has been reported to be better tolerated by man (9) and generally more potent in intact cells and organisms (13). The N⁶ 2'-O-dibutyryl cyclic 3 :5'-adenosine monophosphate monosodium salt was purchased from Sigma Chemical Co St Louis, USA and prepared for human use. A solution (5 mg per ml) was made in 0 15 M NaCl adjusted to pH 7 4 with 0 01 M NaOH and sterilized by filtration on Millipore Filter GS pore size 0.22 μm (Millipore Corp Bedford, Mass. USA) The cyclic nucleotide was infused after an overnight fast at a rate

Table 2 *Peak plasma growth hormone levels obtained in the hypopituitary and Silver Russell patients by different stimuli*

Patients	Peak plasma GH (ng/ml) following stimulation by Insulin	Arginine	Glucagon
Hypopituitary			
C. D	0	0	0
P. D	0	1.3	0.5
M. V	0	0.1	0.2
R. B	<1.0	<1 0	0
H. B	0.4	<1 0	0
A. N	0.8	0.3	0.3
E. G	0 4	0.5	0
R. R.	1 1	1.3	0 4
Silver Russell			
K. S	10	23	48.3
V. M.	>40	15 9	32.2

DIBUTYRYL CYCLIC 3 ,5 ADENOSINE MONOPHOSPHATE IN HYPOPITUITARISM AND SILVER RUSSELL SYNDROME

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ABSTRACT Vanderschueren-Lodeweyckx M. Van den Berghe G Proesmans, W Corbeel L. Eggermont, E. and Eeckels, R. (Department of Paediatrics Katholieke Universiteit Leuven, Belgium) Dibutyryl cyclic 3 ,5 -adenosine monophosphate in hypopituitarism and Silver Russel syndrome *Acta Paediatr Scand 63: 364 1974*—The effect of the infusion of dibutyryl cyclic 3 ,5'-adenosine monophosphate (0.2 mg/kg/min during 1 hour) on plasma growth hormone and on glucose immunoreactive insulin and cortisol was studied in 3 control children, in 2 Silver-Russell patients and in 8 patients with idiopathic hypopituitarism Upon the infusion of the cyclic nucleotide plasma glucose and immunoreactive insulin increased markedly An increased level of plasma cortisol was also observed in all cases except in one hypopituitary patient with associated ACTH deficiency In contrast to what has been reported in normal subjects and observed in the 3 normal children and the 2 Silver Russell patients, dibutyryl cyclic 3 ,5 -adenosine monophosphate failed to increase the level of plasma growth hormone above 1 ng per ml in 7 out of the 8 hypopituitary patients. Exogenous dibutyryl c-AMP may be considered as an alternative test for the study of growth hormone release

KEY WORDS: Cyclic AMP growth hormone blood glucose insulin, cortisol, hypopituitarism, Silver Russell syndrome

Cyclic 3 ,5 adenosine monophosphate (c AMP) is the second messenger of a large number of hormones including the hypothalamic releasing hormones (13) The evidence that the action of growth hormone releasing hormone (GH RH) is mediated by c AMP as first suggested by Schofield (16) can be summarized as follows 1 in pituitary glands *in vitro* hypothalamic extracts provoke an increase in the level of the cyclic nucleotide (20) and parallel release of growth hormone (17) 2. c AMP or its derivatives have been shown to stimulate the release of growth hormone (GH) *in vitro* (10) as well as *in vivo* not only in animals (2) but also in human adults (8 9)

The present studies were undertaken to investigate the effect of exogenous dibutyryl c AMP on plasma GH levels in hypopituitary growth retardation

PATIENTS

As shown in Table I the investigations were performed in 3 control subjects with normal height and weight and without endocrine diseases in 2 Silver Russell patients and in 8 patients with idiopathic hypopituitarism All subjects were prepubertal The hypopituitary patients R. B and H B are siblings and were already

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hypopituitary patients is summarized in Fig. 3 Blood glucose rose in all patients and the peak of the means was 175 1±36.4 mg/100 ml (M±1 S D) Although the increase of blood glucose in these patients was comparable to that observed in the other control subjects and Silver-Russell patients IRI increased to a lesser extent the peak of the means was 52.6±25 5 μU/ml(M±1 S D) No difference in the response between treated and untreated hypopituitary patients was noticed The rise of plasma cortisol occurred in all patients except in case H. B who is presumably ACTH deficient In 7 out of the 8 cases, plasma GH did not rise above 1 ng/ml throughout the test In patient A. N however plasma levels of 2.2 ng/ml at 120 minutes and of 5 0 ng/ml at 135 minutes were recorded.

DISCUSSION

Our results show that dibutyryl c AMP increases plasma GH levels in control subjects and in Silver Russell patients but fails to do so in seven out of eight hypopituitary patients

According to present knowledge the mechanism of GH release involves the three following steps (I) secretion of GH RH by the hypothalamus (II) recognition by the membrane receptor of the somatotrophic cells of the pituitary gland and formation of c AMP from ATP by the membrane-bound adenyl cyclase (III) secretion of GH presumably by exocytosis of preformed granules containing GH The effect of exogenous dibutyryl c AMP on GH release *in vivo* may be hypothetically explained by two different mechanisms Either the dibutyryl c AMP intervenes directly at the above mentioned steps or it triggers the hypothalamo-pituitary system indirectly possibly through its influence on blood glucose In keeping with the first hypothesis is the known *in vitro* effect of c AMP and its analogues on isolated hypophyseal glands (10) However the observed delay of GH secretion after dibutyryl c AMP infusion is more in accord with the second hypothesis It is also conceivable that both mechanisms occur and that *in vivo* the direct action of exogenous dibutyryl c AMP on the pituitary gland is blocked by the early appearing hyperglycaemia.

Patient A. N deserves special comment he is the only patient with hypopituitarism reacting on exogenous c AMP with elevation of plasma GH Although the peak plasma level of 5 0 ng/ml is only at the admitted lower normal level after insulin administration (6) and markedly inferior to the levels observed in the normal children and Silver Russell patients it clearly exceeds the values observed in this child after all other stimuli including insulin arginine and glucagon (Table 2) It is impossible to say however whether or not the release of GH shown by patient A N indicates that the physiopathological mechanism of his GH

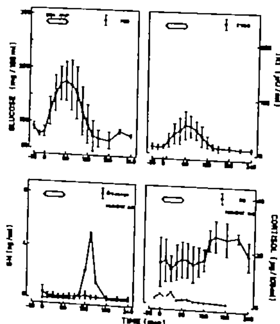


Fig 3 Effect of dibutyryl cyclic 3 5 -adenosine monophosphate (DBc AMP) upon blood glucose immunoreactive insulin (IRI), growth hormone (GH) and cortisol in the hypopituitary patients. The mean ±1 S.D. is shown. A different scale for plasma growth hormone is used.

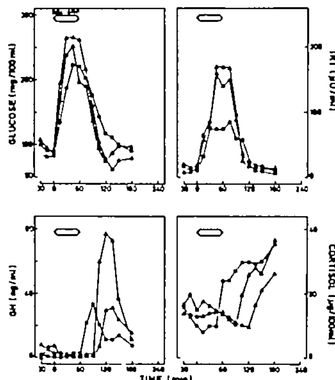


Fig 1 Effect of dibutyryl cyclic 3 5 -adenosine monophosphate (DBc AMP) upon blood glucose immunoreactive insulin (IRI) growth hormone (GH) and cortisol in three normal children Symbols used indicate subject B B (▲) B E (Δ) or R B (■)

of 0.2 mg per kilogram body weight per minute for 1 hour as outlined by Levine (8) In the patients receiving HGH a delay of 3 days after the last injection was observed Minimal side effects were noticed in 6 children, two presented nausea and four vomited The other children had no discomfort Blood samples were collected before the test and every 15 minutes afterwards from a peripheral vein into heparinized tubes cooled at 4°C Plasma was separated by centrifugation and stored at −20°C until assayed Plasma GH was measured by radioimmunoassay with the use of the double antibody method (15) The highly purified HGH (HS 1394) used as standard was a gift of Dr A E. Wilhelmi The guinea pig antihuman GH serum was kindly supplied by Dr R Yalow and the late Dr S A Berson All determinations were carried out in duplicate and sequential samples of the same subject were measured simultaneously Blood glucose was measured by the glucose-oxidase method (14) plasma cortisol by competitive protein binding (11) and insulin by radioimmunoassay (4)

RESULTS

In order to assess the activity of the infused dibutyryl c AMP GH as well as a number of parameters known to be influenced by the cyclic nucleotide (7–9) were followed Fig 1 shows the effect of the dibutyryl c AMP infusion upon plasma GH glucose immunoreactive insulin (IRI) and cortisol in the 3 control children Blood glucose values rose sharply and the maximal values were observed about 45 minutes after the onset of the infusion The release of IRI parallels blood glucose Plasma cortisol increased after the end of the infusion up to the end of the test Plasma GH shows a marked increase following the infusion of the cyclic nucleotide and maximal values respectively of 31 0 ng/ml 77 2 ng/ml and 33 0 ng/ml were observed 135 120 and 90 minutes respectively after the onset of the infusion Fig 2 represents the effect of the dibutyryl c AMP infusion upon the same parameters in the two Silver Russell patients The same patterns were observed as in the control subjects Peak plasma GH levels of 72 3 ng/ml and of 71 5 ng/ml were attained 120 minutes after onset of the infusion in the 2 Silver Russell patients The effect of the dibutyryl c AMP infusion in the

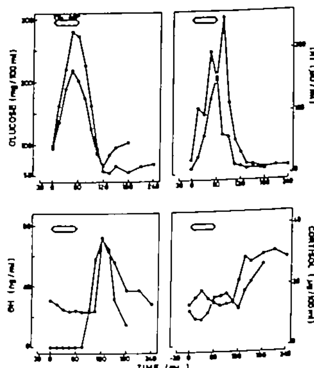


Fig 2 Effect of dibutyryl cyclic 3 5 -adenosine monophosphate (DBc AMP) upon blood glucose immunoreactive insulin (IRI) growth hormone (GH) and cortisol in 2 Silver Russell patients. Symbols indicate the patient K. S (●) and V M (O).

Acta Pædiatr Scand 63 369–372 1974

TEN CASES OF SO-CALLED LONG SURVIVAL IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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ABSTRACT Armata, J Cyklis, R. and Wyszkowski, J (Institute of Paediatrics, Medical Academy in Cracow Cracow Poland). Ten cases of so-called long survival in children with acute lymphoblastic leukemia. Acta Paediatr Scand 63: 369 1974.—Ten cases of acute lymphoblastic leukemia are presented, in children all of whom survived more than 4 years. WBC and blast cell counts were registered at low levels at the onset of the disease and at subsequent bone marrow relapses. Extramedullary leukemia occurred after 2 years (average) in bone marrow remission, which did not predict early bone marrow relapse. From 1962 to 1970 three treatment regimens for acute leukemia were compared; the best results depended on the intensity of treatment during the first remission.

KEY WORDS: Acute lymphoblastic leukemia, long survival, children

Survival for more than 4 or 5 years is considered as "long survival" in acute leukemia (2, 3 21) Long survivals occur most often in children with acute lymphoblastic leukemia (2 3). Acute leukemia may be not an incurable disease in all cases If the first remission lasts for several years recovery becomes possible (3 12)

CASE REPORTS

At the Second Pediatric Clinic, Cracow during the period Jan. 1 1962 to Nov 6, 1968, a total of 95 children under 13 years of age have been treated for acute lymphoblastic leukemia. Survivals for over 4 years occurred in 6 boys and 4 girls. Four children died.

In all children but one the initial symptoms lasted several weeks. The white blood cell (WBC) count in the peripheral blood exceeded 10000/mm³ in 2 cases only (Table 1). The blast—cell count exceeded 500/mm³ twice.

Diagnosis At our Clinic a diagnosis of acute lymphoblastic leukemia is established when paralymphoblasts predominate in the bone marrow Because some of the films were poorly preserved it was impossible to determine subdivision of acute lymphoblastic leukemia (18), although the diagnosis of acute lymphoblastic leukemia was not in doubt

Chemotherapy procedure So-called VAMP (Vincristine Amethopterin, Mercaptopurine Prednisolone) therapy was administered in one child (case 7). In 2 children remissions were obtained after prednisone alone (cases 2 and 4), and in the remaining patients prednisone was given with 6-mercaptopurine. All the children received a low-protein diet (10). Treatment during the first remission was variable.

In the years 1963–65 4 children were treated less intensively during the first remission (Table 2, cases 1 3 4 and 9). S. B. was treated at first in another hospital, but received no drugs during the first remission. These children (cases 1 3 and 4) died during the late relapses of the disease. One child (S B) died during the second remission of the disease from fulminant viral hepatitis. Only one child is still living (case 9). In mentioned children the duration of the first haematological remissions varied from 17 1/2 to 59 months.

Five children, in whom acute leukemia was diagnosed in a later period (1966–68), besides 6-mercaptopurine (MP), methotrexate (MTX), cytoxan (CY) received other drugs in the late phase of the first remission (Table 2, cases 7 and 10) or from the beginning of the remission (cases 5 6 and 8). All 5 children are still living. Duration of the first haematological remissions varied from 30 to 60 months.

Extramedullary involvement of leukemia was observed in 7 children (Table 3) in the late stage of the disease (mean 27 months) mainly in the central nervous system and in the testes, kidneys, salivary glands, lymph

deficiency is different from that of the other patients The answer to that question depends on the hitherto unknown step(s) in GH secretion at which dibutyryl c AMP intervenes *in vivo*

Our observations show that exogenous dibutyryl c AMP may be considered as an alternative test for studying GH release in normal and hypopituitary children It is not impossible that together with the future availability of synthetic GH RH exogenous c AMP will contribute to pinpoint the critical steps of GH release and the defect in patients with GH deficiency

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Table 3 *Duration of the first remission (in months) in relation to the appearance of extramedullary leukemia*

		Involvement of					
Case	Initials	CNS	Kidneys	Testes	Lymph nodes	Salivary Glands	Spleen
1	S. K.	–	–	–	–	–	–
2	S B	–	–	27 0	–	–	–
3	R. U	23.5	–	–	–	–	31 0
4	Z. J	–	–	–	–	–	–
5	W J	11 0	37.5	–	–	–	–
6	G H.	26.0	–	38 0	–	–	–
7	B H.	71.5	–	–	28.0	14 0	–
8	M S.	26.0	–	–	–	–	–
9	S. S	–	–	–	–	1 0	–
10	Z. R	–	–	–	–	–	–

It has also been assumed that low pre-treat ment peripheral blastaemia in children with acute leukemia of long duration might well indicate early diagnosis of the disease (4)

Our cases were characterized by a long period of initial symptoms as well as by low WBC and low blast-cell counts in the peripheral blood before treatment. Slow development of the disease manifesting itself in low WBC counts seems to be a distinctive feature in our cases The two last-mentioned symptoms of acute leukemia were regularly observed also at the onset of bone marrow relapse.

In the observed cases extramedullary involvement occurred after 2 or more years average during bone marrow remission Extramedullary involvement occurring once or even more times (e g. three times in kidneys) as distinct from the usually observed facts, did not interrupt the bone marrow re mission The fact that extramedullary involvement did not as a rule predict rapid bone marrow relapse seems likewise to be a peculiarity of the observed cases and/or the disease itself It has been suggested that allergic constitution (4) as well as normal levels of IgM immunoglobulins (4) predispose to long survivals. Other investigators however found no difference in immunologic reactions between cases of acute leukemia with long survivals and others (2)

The most important factor of long sur vivals is the intensity of treatment during the first remission in acute leukemia (2 13 21 22, 27) At centres where acute lymphoblastic leukemia is treated intensively long survivals occur in 15–25% of patients (2 22) A comparison of the frequency of long first remissions in different periods of time provides a basis for the prediction of whether long survivals in acute leukemia will be more frequent in subsequent years (21 27) In our own material we have compared the therapeutic results in 115 cases of acute leukemia treated in different periods of time (Table 4) in which the children in the first remission were treated by different methods and with increasing intensity In the period 1962–65 as described above treatment during remission was less intensive In the next 3 years in some of the children in first remission 6-

Table 4 *Survival in acute lymphoblastic leukemia in years 1962–70*

		Survival >2-yr		Survival >4-yr	
Date of onset	No. of pats.	No. of pats.	% pats.	No of pats.	% pats.
1962–65	66	17	25 7	5	7.5
1966–68	29	12	41	5	17
1969–70	20	12*	60	?	?

* In 9 children the first bone marrow remission is still lasting.

Table 1 *Initial WBC inducing agent and time of survival*

Case	Patient	Age at start of treatment (years)	Initial WBC count	Drug used for initial induction[b]	Survival (months)
1	S K	7	3300	P MP	66
2	S B	4	7800	P	51½
3	R U	2½	7500	P MP	59
4	Z J	3	11600	P	62
5	W J	5½	12050	P MP	58½
6	G H	6	9700	P MP	51
7	B B	5	3100	P MP VCR MTX	74*
8	M S	5	7200	P MP	48½
9	S S	11	4100	P MP	60*
10	Z R.	5	4800	P MP	68½

Still under treatment
[b] Abbreviations see Table 2

nodes and spleen Extremedullary involvements in acute leukemia were treated with roentgentherapy and MTX injected intrathecally (in meningeal leukemia) or roentgenotherapy and l-asparaginase intravenously (in tumours of the kidneys and testes)

WBC count It was reported previously that blastic hyperleukocytosis at the beginning of treatment for acute leukemia has a tendency to recur at the onset of bone marrow relapse (1)

The cases under consideration were characterized by low WBC and low blast-cell counts in the peripheral blood before treatment The behaviour of these two symptoms in bone marrow relapses of acute leukemia was therefore studied The method of recognizing bone marrow relapses, introduced at our Clinic has been reported previously (1)

In 6 children single or numerous bone marrow relapses occurred A total of 37 relapses was observed At the onset of relapse WBC counts less than 10000/mm³ were observed 35 times For bone marrow relapses a relatively low blast-cell count in the peripheral blood was characteristic

DISCUSSION

Nineteen cases were collected from the literature on long survivals in acute leukemia in which WBC counts before treatment are reported (5–9 11 12 14–17 19 23–26) They were combined with our present series. In 24 out of 29 cases WBC count was less than 10000/mm³ and in 5 cases *higher* The fact that long survivals in acute leukemia are usually characterized by low WBC counts before treatment has been mentioned previously (3 20 21)

Table 2 *First remission*

Abbreviations. MP 6-Mercaptopurine MTX Methotrexate P Prednisone CY Cytoxan VCR. Vincristine DAU Daunorubicin

Case	Patient	Maintenance regimen	Duration of first remission (months)	Current status
1	S K	MP	41	Dead—12th relapse
2	S B	Not treated	19	*Dead—2nd remission*
3	R. U	MP MTX P	50	Dead—6th relapse
4	Z J	MP	17 5	Dead—14th relapse
5	W J	MP MTX CY P VCR DAU	58	Alive—1st remission
6	G H	MP MTX CY P VCR DAU	50	Alive—1st remission
7		MP MTX CY VCR DAU	71	Alive—2nd remission
8	M S	MP MTX CY P VCR DAU	30	Alive—3rd remission
9	S S	MP MTX CY	59	Alive—1st remission
10	Z R.	MP MTX CY VCR	68	Alive—1st remission

Prednisone was given for 1 week before injection of VCR.

Table 3 *Duration of the first remission (in months) in relation to the appearance of extramedullary leukemia*

Case	Initials	*Involvement of* CNS	Kidneys	Testes	Lymph nodes	Salivary Glands	Spleen
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3	R. U	23.5	–	–	–	–	31.0
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5	W. J	11.0	37.5	–	–	–	–
6	G. H	26.0	–	38.0	–	–	–
7	B. B	71.5	–	–	28.0	14.0	–
8	M. S.	26.0	–	–	–	–	–
9	S. S	–	–		–	1.0	–
10	Z. R.	–	–	–	–	–	–

It has also been assumed that low pre-treatment peripheral blastaemia in children with acute leukemia of long duration might well indicate early diagnosis of the disease (4).

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The most important factor of long survivals is the intensity of treatment during the first remission in acute leukemia (2, 13, 21, 22, 27). At centres where acute lymphoblastic leukemia is treated intensively, long survivals occur in 15–25% of patients (2, 22). A comparison of the frequency of long first remissions in different periods of time provides a basis for the prediction of whether long survivals in acute leukemia will be more frequent in subsequent years (21, 27). In our own material we have compared the therapeutic results in 115 cases of acute leukemia treated in different periods of time (Table 4) in which the children in the first remission were treated by different methods and with increasing intensity. In the period 1962–65 as described above, treatment during remission was less intensive. In the next 3 years in some of the children in first remission 6-

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1969–70	20	12*	60	?	?

In 9 children the first bone marrow remission is still lasting.

mercaptopurine methotrexate cytoxan rubidomycin or vincristine were administered with short courses of prednisone Beginning in 1969 and in 1970 children in the first remission were treated as a rule with the six afore-mentioned drugs

The increasing numbers of first remissions lasting longer than 2 years suggests that in the near future remissions lasting longer than 4 years may become more frequent This leads to a more optimistic outlook on the possibility of curing the disease

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EEG FINDINGS IN JUVENILE RHEUMATOID ARTHRITIS AND OTHER CONNECTIVE TISSUE DISEASES IN CHILDREN

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ABSTRACT Lang, H., Anttila, R., Svékus, A. and Laaksonen, Anna-Liisa (Department of Paediatrics and Department of Clinical Neurophysiology University of Turku Turku, Finland). EEG findings in juvenile rheumatoid arthritis and other connective tissue diseases in children. Acta Paediatr Scand 63: 373, 1974.—The object of the study was to determine which factors are most likely to cause EEG changes in children suffering from juvenile rheumatoid arthritis (JRA) and other connective tissue disorders. EEG curves from 100 patients were analysed; 93 of these had JRA, 3 had systemic lupus erythematosus (SLE), 2 psoriasis, 1 dermatomyositis and 1 scleroderma circumscripta.

Pathological EEG was found in 52 patients, borderline in 16 and normal EEG in 32. In the JRA group it was found that if patients had positive antinuclear antibodies or visceral symptoms like pericarditis and/or myocarditis, they had fewer pathological EEG changes than those suffering from a form of the disease with symptoms only in the joints. The number of pathological EEG findings increased with age of the patients.

Pathological EEG changes were found to occur more often in girls than boys, and were connected with those forms of the disease where in addition to symptoms in the joints, there was fever due to the disease itself and possibly also non-infectious leukocytosis.

No correlation was found between EEG changes and the kind of drug treatment of JRA. There was no clear correlation between EEG changes and the duration of the disease or age at onset.

In the group of seven patients with other connective tissue disorders only one with SLE had normal EEG.

The observations give reason to suppose that the pathological EEG changes, especially the slowness of activity and the asymmetrical focal disturbances, are caused by the primary cerebral process connected with the disease itself, probably vasculitis.

KEY WORDS: Juvenile rheumatoid arthritis, EEG

EEG changes are known to take place in 50% of patients with connective tissue disorders, especially with systemic lupus erythematosus and rheumatoid arthritis (9 11) This is true also of juvenile rheumatoid arthritis (JRA) (7) Although it has been thought that these EEG changes have been induced by drugs used in the treatment of the diseases in question (14 4) it is also possible that general EEG disturbances may be caused by the disease itself both in the case of adults and children (11 7)

The object of the present study has been to determine which factors are most likely to cause EEG changes in children suffering from JRA and other connective tissue disorders In order to clarify the etiology of EEG changes an analysis was made of deviations in EEG in one hundred children examined consecutively and these were corre

Table 1 *Sex and age of the patients age at onset and duration of the disease in different diagnostic groups*

		Diagnosis				
		JRA	SLE	Psoriasis	Dermato-myositis	Scleroderma-circumscr
Sex and age of patients						
< 3 yrs	male	5				
	female	16				
3–5 yrs	male	2				
	female	12				
6–10 yrs	male	8	1	1		
	female	28				1
11–16 yrs	male	7	1	1	1	
	female	15				1
Age at onset						
< 3 yrs		34				1
3– 5 yrs		19	1			
6–10 yrs		27	1	1		
11–16 yrs		13	1	1	1	
Duration of the disease						
< 2 mths		4			1	
2– 6 mths		42				
7–12 mths		14	1			
1– 3 yrs		16		1		
> 3 yrs		17	2	1		1

lated with clinical findings laboratory data and drugs administered to the children

MATERIAL AND METHODS

Clinical material

By making systematic recordings from patients who were suffering from JRA or other connective tissue disorders it was possible to collect an unselected series of 100 patients (73 girls and 27 boys). The distribution of the sexes in the material corresponds to that earlier determined for children suffering from juvenile rheumatoid arthritis (8). Table 1 shows the diagnosis sex and age of the patients at the time of EEG recording the age of the patients at onset of the disease in the different disease groups and the duration of the disease

Diagnostic criteria

All patients with JRA fulfilled the following diagnostic criteria (1 3):

1. Joint symptoms with onset before the age of 15
2. Swelling or limited mobility of two or more joints.
3. Continuous presence of joint symptoms for at least 3 months.
4. If only one joint was involved histological synovial study was required for the diagnosis.
5. Elimination of other diseases of joints and connective tissue

Seven of the JRA patients showed a typical Still s form of the disease with septic fever and systemic manifestations. Systemic symptoms had also occurred at some stage of the disease in 5[illegible] other patients (pericarditis myocarditis pleuritis, peritonitis hepatosplenomegaly liver damage kidney damage rash, lymphadenopathy non-infectious fever periods or iridocyclitis).

In one patient amyloidosis had been found with the help of rectum and kidney biopsy

At the time of examination 64 patients were found to be at Stage I 27 at Stage II and 9 at Stage III Thirty-three patients were in Class I 61 in Class II and 6 in Class III (1[illegible])

In looking for other possible factors causing EEG abnormalities the patients anamnestic data were studied in relation to the possible premature birth, mother s toxemia other complications during pregnancy or delivery asphyxia after birth possible icterus in the period immediately following birth, convulsions during fever other anamnestic convulsions and possible psychic disturbances

Drugs administered to patients

Antimalarial treatment for over 6 months ([illegible]4 patients)
Corticosteroid treatment for over 3 months (15 patients)
Phenylbutazone (26 patients)
Gold treatment for over 3 months (15 patients)
Salicylates at the moment of EEG-recording (35 patients)
Combined treatment (37 patients)

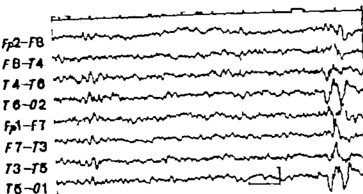


Fig. 1. K. T. 6 yrs 2 months. EEG 258/19.3.70 U. JRA. An example of diffuse EEG-slowing combined with abundant beta-activity, episodic slowing and one generalized irregular spike and slow wave paroxysm. Calibr. 1 sec. 100 μV.

EEG recording

EEG recordings were generally carried out with a 16-channel Elema ink-jet recorder, model Mingograf E M 160/16 A. In placing the electrodes, the international 10–20 system was applied, and both bi-polar and common reference derivations were used. The recordings were made by an experienced EEG technician in the clinical neuro-physiological department of the hospital. Photic stimulation was used with all patients and effectual hyper-ventilation was achieved in the case of approximately a quarter of the patients and sleep recording in the case of about half. The material contains in all 123 EEG curves, the reason being that in the case of 12 patients the recording was made two or more times usually because there was a clear abnormality in the first curve or a change in the clinical picture of the disease.

Two specialists with many years' experience of children's EEG curves first judged the curves independently of one another, without knowing the clinical details, and they marked the results digitally on a form. Those curves which had been differently classified were then studied by both specialists together. Clinical, laboratory and EEG data were then transferred to punch cards and correlation calculations were carried out by the Calculation Centre of Turku University applying Chi Square and Kendall' four square tests.

For the correlation analysis, activity was classified as slow (range 0–3), focal/asymmetrical (range 0–2) and paroxysmal (range 0–2) (spikes, sharp waves or spike and slow wave complexes, either as asymmetric manifestations or as bilateral or generalized discharges).

Only moderate or marked slowness which could not be explained on the basis of drowsiness, or hyper-ventilation connected with crying was interpreted as abnormal and classified as slow cortical electrical activity independently of the more exact quality of the disturbance. Photic stimulation and hyper-ventilation fairly frequently had the effect of emphasizing the slow activity but because, especially in the case of small children, hyper-ventilation is difficult to carry out effectively and because flashlight often causes drowsiness only paroxysmal or asymmetric disturbances by activation were taken into account.

RESULTS

1. *EEG findings*

Out of the 100 cases studied, 32 patients had a normal EEG, there were 16 borderline cases and 52 had abnormalities. In a number of curves several different types of disturbance appear.

Slowness of activity was the commonest type of disturbance, but it varied greatly in degree. At least moderate slowness of activity occurred in 34 patients. The slowness varied from continual to episodic and concerned either the occipital rhythm or diffuse background activity or both.

Beta-activity appeared more abundantly than usual in 27 out of 123 curves, but the phenomenon correlated clearly with the slowness of activity: only in 6 cases was there abundant beta-activity without clear simultaneous slowness of activity.

Fig. 1 shows an example of a common diffuse EEG abnormality.

Focal-asymmetrical disturbances, either *paroxysmal* or *non-paroxysmal*, occurred in altogether 38 cases in the consecutive material of 100 curves, but in most cases the disturbance was slight and/or occurred occasionally. In 13 cases the finding was very clear or could be shown in successive recordings. In 2 of the latter cases the focal disturbance was formed by spike activity. In other cases the focal disturbance consisted of slow waves, sometimes rhythmic, and of inter

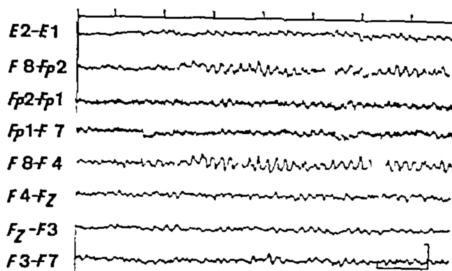


Fig 2 P T 13 yrs 7 months. EEG 184/8 10.70 U JRA. An example of intermittent asymmetric theta-rhythm in the right frontotemporal region Calibr 1 sec 100 μV

mittent type (Fig 2) In 2 cases a continuous rather arrhythmic focal activity of delta/theta frequency was discovered (Fig 3) The slow focuses were rather often connected with general slowness in varying degree if we also take into account mild cases focality /asymmetry and slowness were combined in 32 cases

Five children formed a category of their own in which were seen groups of complexes formed by slow waves and a sharp transient symmetrically located at the vertex This phenomenon differed clearly in its morphology from the vertex waves although it was always manifested during light sleep The average age of these children 2 years 8 months was considerably lower than the average age for the whole material In other respects focuses or asymmetries did not correlate with the age of the children

Bilateral paroxysmal disturbances occurred altogether in twelve curves out of the 100 in the consecutive material The morphology of the disturbances varied greatly In about half the cases in this group there were generalized but irregular spike and slow wave paroxysms (Fig 1) in 2 cases there were 6 Hz spike and slow wave paroxysms In some

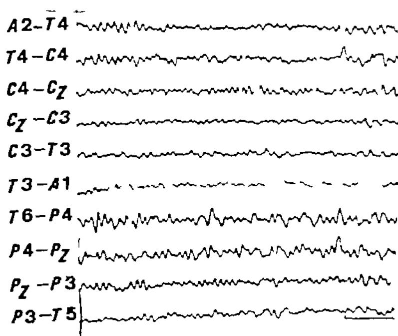


Fig 3 L. J 6 yrs 8 months. EEG 919/12 10 70 U JRA An example of continuous, irregular slowing at right. Calibr 1 sec, 100 μV

ble 2 *General normative EEG classifica-
on and different diagnostic groups*

agnosis	Normal	Border line	Pathological
A	31	12	50
E	1	2	0
ormatis	0	1	1
rmatomyositis leroderma	0	1	0
rcomcripta	0	0	1

ses the disturbances were partial and were ited to the occipital or central regions or ey preceded generalized paroxysms

Bilateral paroxysms often occurred or increased during sleep and/or in flashlight. otic-induced paroxysms were seen in 7 ses (5 girls and 2 boys) Hyper ventilation l not cause paroxysms in a single case The roxysms either spontaneous or induced by ep/flash light never represented the typi- 3 Hz spike and slow wave pattern The ration of bilateral paroxysmal disturbances s generally of the order of 1–2 seconds d there were no clinical manifestations nnected with them. Bilateral disturbances re combined in the most varied way with neral and local disturbances only in 2 ses was the bilateral disturbance the sole ding. The average age of the population scribed was 6 years 9 months (range 3 years onths to 11 years 9 months)

*Correlation of EEG findings with
ical and laboratory data*

ble 2 shows the distribution of EEG finds in different diagnostic groups.

Of 7 patients suffering from the Still s m of the disease 4 were pathological one s borderline and 2 had a normal EEG

When studying the group of 52 patients o at some stage of the disease had had stemic manifestations and comparing it h the group which had no systemic sympns it could be observed that in both groups one-third of the patients had a nor mal EEG

When studying the correlation between different systemic symptoms and pathological EEG changes (Table 3) it was observed that in those patients who had had periods of non-infectious fever resulting from the disease itself there was an increase in the slowness factor in EEG ($p<0.05$) The paroxysmal factor was also increased in cases with fever but the difference was only slight

In patients where myocarditis or pericarditis had been diagnosed there were fewer pathological EEG changes than in the patient material as a whole The difference in these cases was nearly significant ($p<0.05$)

In order to determine the causal relation ship between *fever* and EEG changes the effect of fever on EEG abnormalities at the moment of recording was studied The result is seen in Table 4 In cases where fever re sulted from infection the number of pathological EEG changes was clearly smaller than

Table 3 *EEG findings and systemic manifestations*

Systemic manifestations	No of patients	Correlation to the pathological EEG findings
Non-infectious fever periods	32	Increase in the slowness factor ($p<0.05$) Paroxysmal factor slight increased ($p<0.1$)
Myocarditis	15	Fewer pathological EEG changes than in the material as a whole ($p<0.05$)
Pericarditis	4	Fewer pathological EEG changes than in the material as a whole ($p<0.05$)
Pleuritis	2	No
Peritonitis	1	No
Rash	10	No
Hepatosplenomegaly	6	No
Lymphadenopathy	6	No
Liver damage	1	No
Kidney damage (transient)	31	No
Myopathy	2	EEG pathological
Meningoencephalitis	3	EEG pathological
Amyloidosis	1	EEG normal
Iridocyclitis	15	–

Table 4 *General normative EEG classification and fever at the moment of recording*

1 Fever as systemic manifestation of the disease (32 patients)

(*a*) Fever at the moment of recording (27 patients) / (*b*) No fever at the moment of recording (5 patients)

EEG	(a) *n*	(a) %	(b) *n*	(b) %
normal	7	26	–	–
borderline	4	15	1	20
pathological	16	59	4	80

2 Sub-febrile temperature (<38°C) at the moment of recording (28 patients)

EEG	*n*	%
normal	6	21
borderline	4	15
pathological	18	64

3 Fever resulted from infection (14 patients)

EEG	*n*	%
normal	10	71
borderline	1	8
pathological	3	21

4 No fever (26 patients)

EEG	*n*	%
normal	8	31
borderline	4	15
pathological	14	54

in cases with no fever and cases where the fever or sub-febrile temperature (<38°C) were due to the disease itself

In studying the stage of the disease in patients suffering from JRA and their functional capacity it was discovered that the Stage or Class category did not correlate with EEG changes

EEG findings were normal in the case of the patient suffering from amyloidosis

Three patients had earlier suffered from meningo-encephalitis and they all showed pathological EEG The etiology of meningo-encephalitis remained obscure in these cases there could be question of a process caused by the collagen disease

Positive anamnestic data concerning other possible factors causing EEG abnormalities (premature birth mother s toxaemia, etc)

Table 5 *General normative EEG classification and the sex of the patients*

Sex	Normal *n*	Normal %	Borderline *n*	Borderline %	Pathological *n*	Pathological %
Boys	14	51 9	4	14 8	9	33.3
Girls	18	24 7	12	16 4	43	58.9
	32		16		52	

$p<0.05$

were found in 16 patients but in these cases however fewer than average pathological EEG changes occurred

With regard to the sex of the patients it was observed that girls more frequently showed pathological EEG than boys and the difference was nearly significant ($p<0.05$) (Table 5) The relatively greater prevalence of EEG abnormalities among girls was not due to the asymmetry of the sex distribution and occurred independently of the classification of borderline cases in the Kendal test as normal or pathological The difference between the sexes however concerned only the general normative EEG classification

The number of pathological findings increased with age (Table 6) A significant ($p<0.05$) correlation between age and paroxysmal phenomena was found but on the other hand not between age and focal-asymmetrical phenomena There was no clear correlation between EEG changes and the duration of the disease or age of onset of the

Table 6 *General normative EEG classification and the age of the patients*

Age (years)	Normal *n*	Normal %	Borderline *n*	Borderline %	Pathological *n*	Pathological %
< 3	13	61 9	2	9.5	6	28.6
3– 5	4	28 6	2	14.3	8	57 1
6–10	9	23 1	5	12 8	25	64 1
11–16	6	23 1	7	26 9	13	50.0
	32		16		52	

$p<0.05$

Table 7 *Laboratory findings and pathological EEG changes*

Laboratory findings	No of pats	Correlation to the pathological EEG changes
ESR > 90 mm/h	39	–
Hb < 10 g/100 ml	26	–
Non infectious leukocytosis	7	Slowness factor increased ($p<0.05$)
Positive CRP	28	–
Positive antinuclear factor (ANF)	47	Less than average EEG changes ($p<0.05$) (especially focal asymmetric findings)
Positive cryoglobulins	14	–
Positive latex	22	–
Positive Waaler Rose (≥1 64)	15	–

disease In studying the correlation between laboratory findings and pathological EEG changes (Table 7) it was observed that if the patient had a considerable noninfectious *leukocytosis* the slowness factor had increased in EEG ($p<0.05$)

The antinuclear antibodies were positive in 47 patients and in these patients less than average EEG changes occurred especially when taking into account changes in focality-asymmetry ($p<0.05$)

The effect of drug treatment on EEG

Twenty-two patients had received no drug treatment at all and of these 15 had pathological EEG In separate studies of the effect on EEG of antimalarial drugs gold corticosteroids phenylbutazone salicylates and different combinations of these drugs no correlation was found between EEG changes and the kind of treatment No correlation was found either between the duration of particular drug treatment and EEG changes

DISCUSSION

Twelve of the patients in the material had bilateral spike and slow wave paroxysms and 2 showed a clear spike focus Photic-induced bilateral paroxysmal activity was seen in 7 cases (5 girls and 2 boys) This corresponds to the prevalence figures given in the large normal material of Eeg-Olofsson (5) and age distribution is approximately the same in both materials The influence of age on EEG findings may partly be explained by the fact that an abnormality in EEG is easier to detect as the electrical activity in the brain becomes more mature In the Eeg-Olofsson s (5) material there was also a positive correlation between paroxysmal phenomena and age a correlation that was found in our material Thus the disease itself was not necessarily the cause of the paroxysmal EEG changes.

However approximately one–third of the patients in this material showed in EEG varied slowness of activity and approximately 40% showed asymmetrical-focal EEG changes about half the latter being of the equivocal or unequivocal paroxysmal type The great number of abnormal or borderline cases found in this material corresponds almost exactly to the figures in the material of Kozma & Bacia (7) comprising 50 JRA patients

Jan et al (6) suggest that the reason for cerebral complications and EEG changes in the acute phase of JRA could be toxic encephalopathy The positive correlation between fever and leukocytosis and EEG slowness and paroxysmal phenomena gives reason to suppose that the EEG changes mentioned are the result of hyperthermia and secondary metabolic effects on the child s central nervous system connected with it It is known that artificially induced hyperthermia causes a slowing down of the cortical activity in children and even spike and slow wave paroxysms (2) In some follow-up studies although unsystematically carried out we did discover EEG normalization after the acute phase

On the other hand in those children who had a fever resulting from a secondary infection there were pathological EEG findings less often than in the case of those whose

Table 4 *General normative EEG classification and fever at the moment of recording*

1 Fever as systemic manifestation of the disease (32 patients)

(*a*) Fever at the moment of recording (?7 patients)

EEG	*n*	%
normal	7	?6
borderline	4	15
pathological	16	59

(*b*) No fever at the moment of recording (5 patients)

EEG	*n*	%
normal	–	–
borderline	1	?0
pathological	4	80

2 Sub-febrile temperature (<38°C) at the moment of recording (28 patients)

EEG	*n*	%
normal	6	21
borderline	4	15
pathological	18	64

3 Fever resulted from infection (14 patients)

EEG	*n*	%
normal	10	71
borderline	1	8
pathological	3	?1

4 No fever (26 patients)

EEG	*n*	%
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in cases with no fever and cases where the fever or sub-febrile temperature (<38°C) were due to the disease itself

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Positive anamnestic data concerning other possible factors causing EEG abnormalities (premature birth mother s toxaemia etc) were found in 16 patients but in these cases however fewer than average pathological EEG changes occurred

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Table 5 *General normative EEG classific tion and the sex of the patients*

Sex	Normal		Borderline		Patholog[ica]	
	n	%	*n*	%	*n*	%
Boys	14	51 9	4	14.8	9	33.3
Girls	18	24 7	1?	16.4	43	58 9
	32		16		52	

$p<0.05$

Table 6 *General normative EEG classification and the age of the patients*

Age (years)	Normal		Borderline		Pathological	
	n	%	*n*	%	*n*	%
< 3	13	61 9	?	9 5	6	28.6
3– 5	4	28 6	?	14 3	8	57 1
6–10	9	23 1	5	12.8	25	64 1
11–16	6	‹3 1	7	?6 9	13	50 0
	32		16		5?	

$p<0.05$

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THE CLINICAL USEFULNESS OF C-REACTIVE PROTEIN (CRP) DETERMINATIONS IN BACTERIAL MENINGITIS AND SEPTICEMIA IN INFANCY

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ABSTRACT. Sabel, K.-G. and Hanson, L. Å. (Department of Paediatrics, Children's Hospital, and Department of Immunology, Institute of Medical Microbiology, University of Göteborg, Sweden). The clinical usefulness of C-reactive protein (CRP) determinations in bacterial meningitis and septicemia in infancy. Acta Paediatr Scand, 63: 381, 1974.—The possibility of using CRP for diagnosis and follow-up of infants with bacterial meningitis-septicemia was investigated. CRP was quantitated with the single radial immunodiffusion technique. In 37 infants 0–12 months old with purulent meningitis and/or septicemia, CRP was increased in 33. The same proportion of increased CRP was found in the neonatal cases as in the older infants. Peak values to >50 μg/ml were found in 2/3 of all cases, *E. coli* infections showing the most uniform pattern of high CRP values. The period of increased CRP was closely related to risk of recurrence of the infection. After CRP had returned to normal levels no recurrence occurred. In cases of neonatal *E. coli* infections CRP was found to be the best single parameter indicating persistence of infection, and in a group of *H. influenzae* infection CRP was as good as cultures of cerebrospinal fluid, white blood cell count and protein in cerebrospinal fluid. The findings show that CRP is a useful parameter to show the presence of meningitis and/or septicemia in infancy including the neonatal period. CRP is an easy test which can be used to direct antibiotic treatment since it rapidly detects persistence of infection or recurrence.

KEY WORDS: C-reactive protein, bacterial meningitis and septicemia, infancy

The well-known increase of CRP in patients with infections, especially bacterial infections, seems to occur also in the neonate (2). Felix et al. (7) have shown that septicemia and bacterial meningitis consistently induce formation of CRP in young infants. In an earlier work we observed that pyelonephritis caused by Gram-negative bacteria caused formation of high amounts of CRP in infants 1–15 months of age (17). These findings suggested the possibility of using CRP for diagnosis and follow-up of patients with bacterial meningitis—septicemia. This would be especially valuable in the neonatal period when clinical and laboratory signs of this condition may be difficult to interpret. Furthermore the follow-up of the patient with bacterial meningitis–septicemia may cause considerable problems since parameters like micro-sedimentation rate (MSR), total white blood count (WBC), content of protein and white cells in the cerebrospinal fluid (CSF) and the clinical picture may be insufficient for judgement.

We wanted to investigate whether or not another parameter such as repeated determinations of CRP could add to the possibilities to judge if the infant had been successfully treated so that antibiotic therapy could be stopped. It was also of interest to try to demonstrate whether or not the CRP level could reflect recurrences with the same or another bacterial strain.

fever probably resulted from the disease itself There is good reason for carrying out follow-up studies in connection with connective tissue disorders and checking of EEG after a sufficiently long fever free period has elapsed in order to exclude with certainty the possible secondary effect of hyperthermia on the central nervous system

Even more difficult to explain on the basis of fever are the rather common focal asymmetrical findings in the EEG of children suffering from JRA These observations also give reason to suppose that at least some of the EEG changes in patients are caused by the primary cerebral process connected with the disease itself probably vasculitis It has been possible to verify with X rays vascular changes in the central nervous system of JRA patients (10) and vascular changes in the central nervous system of JRA patients have also been ascertained histologically at necropsy (13)

The fact that 15 patients were shown to have pathological EEG before the commencement of drug treatment suggests that EEG changes were caused by the disease itself As the disease proceeds the frequency of EEG changes does not increase and this also seems to show that drug treatment is not the usual cause of EEG changes

According to the literature on the subject EEG changes are not greater in SLE than in JRA Our study also showed that patients who were found to have positive antinuclear antibodies had fewer pathological EEG changes than was the case for the whole material Moreover symptoms typical of SLE like pericarditis and myocarditis correlated negatively with pathological EEG changes

ACKNOWLEDGEMENT

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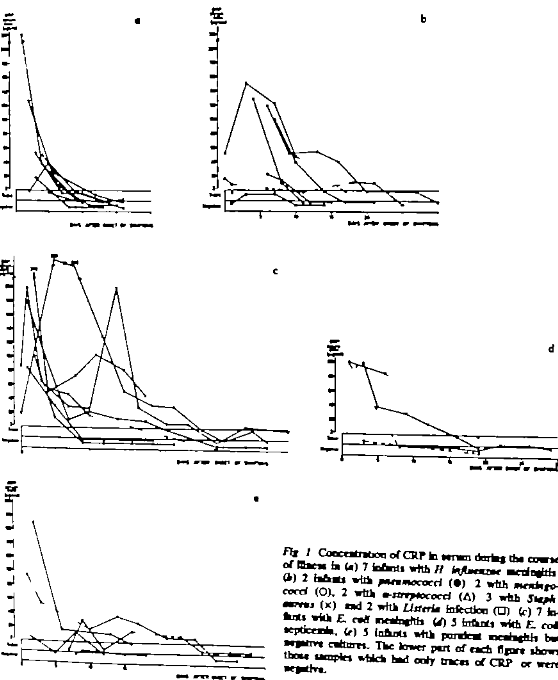


Fig. 1. Concentration of CRP in serum during the course of illness in (*a*) 7 infants with *H. influenzae* meningitis (*b*) 2 infants with *pneumococci* (●), 2 with *meningococci* (O), 2 with *a-streptococci* (Δ), 3 with *Staph. aureus* (×) and 2 with *Listeria* infection (□) (*c*) 7 infants with *E. coli* meningitis (*d*) 5 infants with *E. coli* septicemia, (*e*) 5 infants with purulent meningitis but negative cultures. The lower part of each figure shows those samples which had only traces of CRP or were negative.

The most uniform pattern of high CRP was seen in 11 of 12 *E. coli* infections (range 55–500 μg/ml, $M = 162$ μg/ml) while one case of *E. coli* septicemia and urinary tract infection had only trace amounts. In this case, however, only one sample was obtained at the onset of the disease. The 7 neonatal patients with *E. coli* infection all had high maximal levels of CRP ($M = 198$ μg/ml).

More scattered values were seen in the 20 patients with bacterial infections of other etiology. Six of these were neonatal patients, 2

Table 1 *Cause of infection age of infants and number of patients with septicemia–meningitis*

Cause of infection	No of patients	Age in months at onset of symptoms		
		<1	1–6	7–12
Coliforms	12	7	5	0
Other bacteria	20	6	6	8
Cultures negative				
Purulent meningitis	5	1	4	0
Total	37	14	15	8

MATERIAL AND METHODS

Serum samples were obtained from 37 infants with septicemia or infection of the CNS Thirty two of these had proved bacterial infection with positive cultures from blood and/or CSF All the patients were treated at the Department of Paediatrics Children s Hospital Göteborg and the material was collected during a period of 2 ½ years during which only minor changes were made in the handling of such cases The ages of the patients appear in Table 1 which also shows that 12 of the cases had infections caused by coliforms and 20 by other bacteria such as *H influenzae* (8) *pneumococci* (2) *meningococci* (2) *α-streptococci* (3) *Staph aureus* (3) and *Listeria monocytogenes* (2) Three of the patients with septicemia caused by coliforms also had urinary tract infections Finally there were 5 cases of purulent meningitis without any bacterial findings

The diagnosis and the judgement of the clinical course was based on cultures of blood and CSF as well as follow-up of WBC and MSR and repeated taps of CSF analysed for protein, cells and glucose and finally determinations of the CSF/blood glucose ratio Blood samples for CRP determinations were usually obtained every 3–5 days during the illness Clinical symptoms such as fever or low temperature lethargy or irritability respiratory distress cyanosis jaundice feeding problems vomiting and signs of meningeal irritation such as a tense fontanel stiffness of the neck a positive Kernig sign and convulsions were followed during the course of the illness.

Treatment was instituted as soon as material had been obtained for cultures Two of the cases were on ampicillin before the first cultures were obtained Neonates with suspected bacterial infections were started on a combination of ampicillin and kanamycin and in some cases sulfaisodimidine was added Older infants were started on a combination of penicillin G chloramphenicol and sulfaisodimidine Changes of treatment were made according to the resistance patterns of isolated bacteria. Appearing recurrences were treated in a similar manner

The CRP determinations were performed with the comparative double diffusion-in-gel technique and the single radial immuno-diffusion method as earlier described (8 11) Concentrations of 0.5–1 0 μg/ml of CRP are recorded as trace amounts and higher concentrations are expressed in μg/ml

RESULTS

Occurrence and amount of CRP during infection

During the course of illness CRP was found in all the 37 patients with verified or suspected bacterial infection 32 had maximal values >10 μg/ml whereas five had only traces (Table 2) Peak values ≥50 μg/ml were found in 2/3 of the cases with proved bacterial meningitis or septicemia

Table 2 *Maximum demonstrable CRP in serum in relation to species of bacteria cultured from CSF and/or blood*

Bacteria cultured from CSF and/or blood	No of patients	Max demonstrable C-reactive protein (CRP) μg/ml					
		≥100	99–50	49–25	24–10	Trace	Not present
E coli	12	6	5			1	
H influenzae	8	2	3	1	1	1	
Pneumococc	2	1			1		
Meningococc	2	1			1		
α-streptococc	3	1			2		
Staph aureus	3	2		1			
Listeria	2					2	
Total	32	13	8	2	5	4	0
Purulent meningitis without demonstrable bacteria	5	1	1	1	1	1	
Total with verified or suspected bacterial infection	37	14	9	3	6	5	0

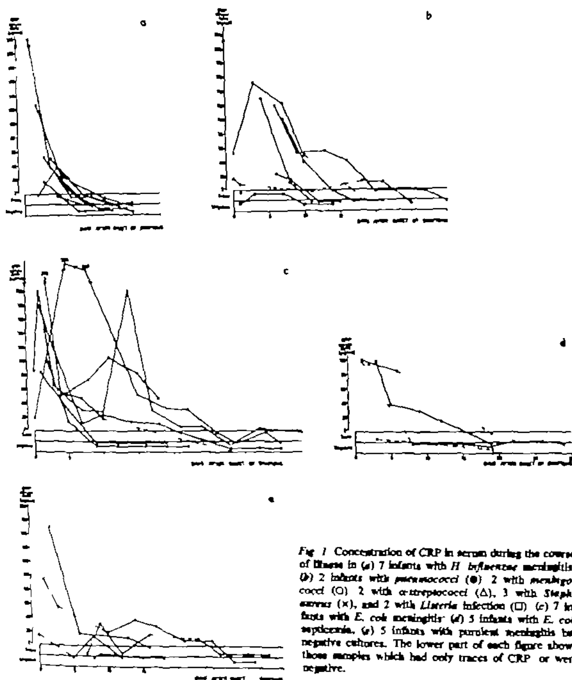


Fig 1 Concentration of CRP in serum during the course of illness in (*a*) 7 infants with *H influenzae* meningitis (*b*) 2 infants with *pneumococci* (●) 2 with *meningococci* (○) 2 with α-*streptococci* (Δ), 3 with *Staph aureus* (×), and 2 with *Listeria* infection (□) (*c*) 7 infants with *E. coli* meningitis (*d*) 5 infants with *E. coli* septicemia, (*e*) 5 infants with purulent meningitis but negative cultures. The lower part of each figure shows those samples which had only traces of CRP or were negative.

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More scattered values were seen in the 20 patients with bacterial infections of other etiology Six of these were neonatal patients 2

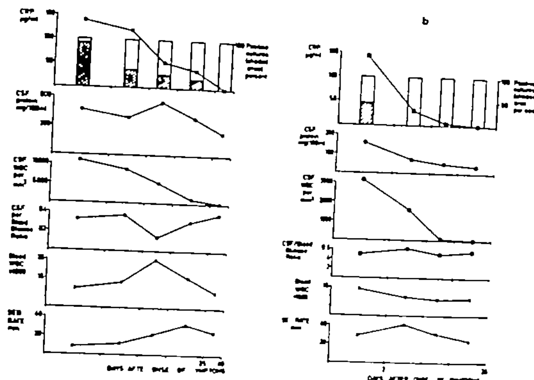


Fig 2 CRP compared with other parameters of infection in (*a*) 7 infants with neonatal *E coli* infections (6 cases with meningitis and 1 case with septicemia only) (*b*) 7 infants 2–11 months old with *H influenzae* meningitis The arithmetic medium of the different parameters obtained during the same periods of time after start of the illness is plotted. The shaded area refers to positive cultures from CSF (in cases with meningitis), and blood (in cases with septicemia solely) as percentage of all cultures obtained during that period of illness.

infected with *Staph aureus* 2 with *α-streptococci* and 2 with *Listeria monocytogenes* These neonates showed variable CRP levels with a maximum of 16–119 μg/ml except the two with *Listeria* infection who had traces only (Table 2)

Of the 5 cases with purulent meningitis where bacterial etiology was suspected but not verified two had high peak values Those two had been treated before cultures were obtained One of these 2 patients was a neonate with CRP 170 μg/ml

The first sample obtained for CRP determination was taken within the first 4 days of illness in 72% of the cases and within 8 days in the rest From 2 of the 5 patients showing traces of CRP the first sample had not been obtained until the fifth and the eighth day

A rapid fall of CRP from the initial values was generally seen during therapy Cases with *H influenzae* infections all became negative or showed traces within the first week of illness (Fig 1 *a*) In some cases of *Staph aureus* infections and meningococcal meningitis a more protracted course was seen (Fig 1 *b*) but this was most obvious in *E coli* infections where 5 of 7 cases with meningitis (Fig. 1 *c*) and at least one of 5 cases with septicemia (Fig 1 *d*) showed a prolonged course with increased CRP levels often with a plateau or secondary peak Some of those cases did not become negative until after 3 weeks or more Two cases of purulent meningitis of unknown etiology with high initial levels of CRP also remained elevated for a long period of time (Fig 1 *e*) Both had been treated with ampicillin before the first CSF culture was obtained

Comparison of CRP with other diagnostic parameters

The relation between CRP and other diagnostic parameters was analysed in detail in the group of 7 neonatal patients infected with *E*

coli and 7 of the infants 2–10 months of age with *H influenzae* It is clearly seen from Figs. 2 *a* and *b* that the levels of CRP closely follow the occurrence of positive bacterial cultures. As long as bacteria can be shown CRP is also demonstrable but turns quickly undetectable after the bacteria have disappeared This is in contrast to other parameters such as CSF/blood glucose ratio CSF protein, blood WBC and MSR which seem much less reliable indicators of the presence of an *E coli* infection. In these cases the WBC in CSF also show a rather good correlation to the presence of bacteria, but in 2 cases normal numbers of WBC were found although bacteria were cultivated from the CSF The CRP was still increased in these 2 patients No recurrence occurred after CRP had returned to normal levels

In the patients with infections caused by *H influenzae* a good correlation was seen between positive cultures and increased levels of CRP WBC and protein of the CSF The three latter parameters all normalised parallel to each other In contrast the CSF/blood glucose ratio and MSR showed a poor correlation to the presence of bacteria.

The usefulness of CRP during infection illustrated by case reports

The correlation between CRP and other parameters of infection and the value of CRP in clinical work is illustrated by three case reports.

Case Reports

Case I

Girl born after 38 weeks gestation, 48 hours after rupture of the fetal membranes. Her mother had no signs of infection. No asphyxia was noted. Birth weight was 2 460 g, length 48 cm She had slight signs of dysmaturity

The day after delivery she was found to have a pale grey colour cyanosis of the hands and feet, tachypnea and slightly elevated temperature A lumbar puncture, a culture from blood and urine and a CRP was taken at 16 hours of age (Fig. 3). Neonatal meningitis was diagnosed and *E. coli* was cultured from blood and CSF urine culture was negative. She was put on ampicillin and kanamycin, and a good clinical recovery was noted. CSF was sterile on the 5th day with WBC falling to zero and the protein level coming back to normal.

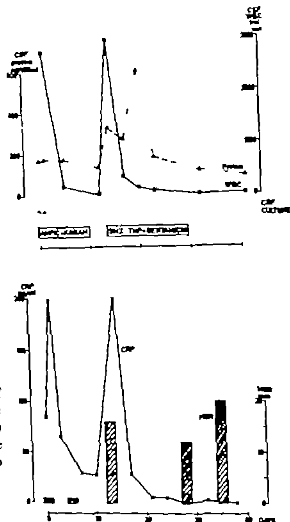


Fig 3 Case I The course of illness as reflected by protein, white blood cells in CSF and cultures from cerebrospinal fluid, as well as C-reactive protein and microsedimentation rate. The periods of antibiotic treatment are indicated. SMZ–TMP = a combination of sulfamethoxazole and trimethoprim.

Treatment with ampicillin and kanamycin was completed after 10 days. Two days later a relapse was obvious, and bilateral subdural effusions were also diagnosed *E. coli* grew again in CSF from lumbar puncture subdural punctures and in blood culture. She was put on a sulfamethoxazole-trimethoprim combination intravenously and a rapid clinical recovery was noted, but *E. coli* was still present in CSF 3 days after the change of therapy Gentamicin was added and given i.m. as well as into the subdural effusions. CSF finally became sterile after 20 days of illness. Repeated subdural taps were made during 4 weeks until no further fluid was obtained. Antibacterial treatment was given until the 32nd day The girl was dismissed from hospital at 7 weeks of age.

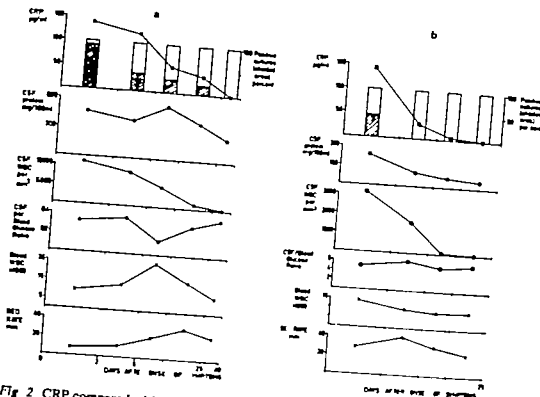


Fig 2 CRP compared with other parameters of infection in (*a*) 7 infants with neonatal *E coli* infections (6 cases with meningitis and 1 case with septicemia only) (*b*) 7 infants 2–11 months old with *H influenzae* meningitis The arithmetic medium of the different parameters obtained during the same periods of time after start of the illness is plotted The shaded area refers to positive cultures from CSF (in cases with meningitis) and blood (in cases with septicemia solely) as percentage of all cultures obtained during that period of illness.

infected with *Staph aureus* 2 with *α-streptococci* and 2 with *Listeria monocytogenes* These neonates showed variable CRP levels with a maximum of 16–119 μg/ml except the two with *Listeria* infection who had traces only (Table 2)

Of the 5 cases with purulent meningitis where bacterial etiology was suspected but not verified two had high peak values Those two had been treated before cultures were obtained One of these 2 patients was a neonate with CRP 170 μg/ml

The first sample obtained for CRP determination was taken within the first 4 days of illness in 72% of the cases and within 8 days in the rest From 2 of the 5 patients showing traces of CRP the first sample had not been obtained until the fifth and the eighth day

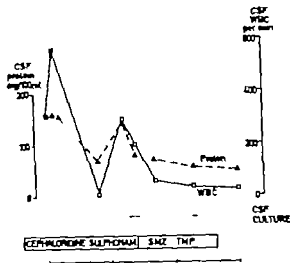
A rapid fall of CRP from the initial values was generally seen during therapy Cases with *H influenzae* infections all became negative or showed traces within the first week of illness (Fig 1 *a*) In some cases of *Staph aureus* infections and meningococcal meningitis a more protracted course was seen (Fig 1 *b*) but this was most obvious in *E coli* infections where 5 of 7 cases with meningitis (Fig. 1 *c*) and at least one of 5 cases with septicemia (Fig 1 *d*) showed a prolonged course with increased CRP levels often with a plateau or secondary peak Some of those cases did not become negative until after 3 weeks or more Two cases of purulent meningitis of unknown etiology with high initial levels of CRP also remained elevated for a long period of time (Fig 1 *e*) Both had been treated with ampicillin before the first CSF culture was obtained

Comparison of CRP with other diagnostic parameters

The relation between CRP and other diagnostic parameters was analysed in detail in the group of 7 neonatal patients infected with *E*

day he again had a temperature rise to 38 6°C, and CSF showed a deterioration by increase of protein content and cell count. The therapy was changed to a combination of sulfamethoxazole and trimethoprim, and a rapid recovery was noted His colour became normal he began to gain weight, and the CSF was returning to normal He was dismissed from hospital after 5 weeks. When last seen at 17 months of age development was quite normal, and he had no sequelae but for a brief general convulsion on one occasion.

CRP in this case Initially a high value of 95 µg/ml was seen (Fig. 5) decreasing to 19 µg/ml on the tenth day when the boy obviously was improving. During the relapse a new peak reaching at least 37 µg/ml was seen, although WBC and protein of CSF showed only moderate increase After change of therapy CRP fell below 10 µg/ml in 1 week and was negative after 2 weeks when therapy was ended. No relapse occurred.



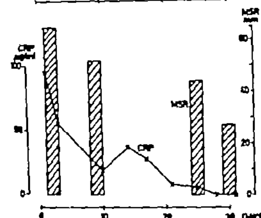


Fig 5 Case 5 See caption to Fig. 3

DISCUSSION

Using sensitive and relatively accurate methods CRP has been shown to be present in small amounts in serum of healthy infants (7 11) being formed already by newborns (1 2, 3 7) and premature infants (2) In a recent study by Kindmark (7) an upper two sigma limit was given of 0.6 µg/ml for healthy newborns, 3.2 µg/ml at the age of 1 day and 1 6 µg/ml at the age of 1 week to 1 month Using a less sensitive method than Kindmark, Saxstad et al (11) found CRP values ranging from 0.5–1 µg/ml to 6 µg/ml in 16% of healthy infants 1–15 months of age

A quantitative determination of CRP in infants 1–15 months of age with various infections was performed by Saxstad et al (12) who found increased amounts in all cases of pyelonephritis and in infants with verified bacterial infections in contrast to infections of viral origin. In the initial stage of pyelonephritis a fast increase of CRP could be demonstrated Felix et al (2) using the less specific and sensitive capillary precipitin test found positive CRP of 2–10 mm in all their cases with septicemia or meningitis. They could demonstrate a positive reaction as early as 2 hours of age in a case of *Staph aureus* infection. Their method was not sensitive enough to be used as a diagnostic guide during the course of illness.

We have studied 37 unselected cases of septicemia and/or purulent meningitis in the first year of life including 14 cases in the neonatal period when the diagnostic and therapeutic problems are most prominent Increased CRP (>10 µg/ml) was found in the same proportion in the neonatal cases (85 7%) as in the older infants (86.9%) Since the CRP samples were taken late in the illness in two of the cases and one sample only was obtained in each of two other cases with traces of CRP this may be considered minimum figures The neonatal Gram-negative infections which cause the greatest therapeutic problems all showed high values

In our series the levels of CRP closely followed the occurrence of positive bacterial

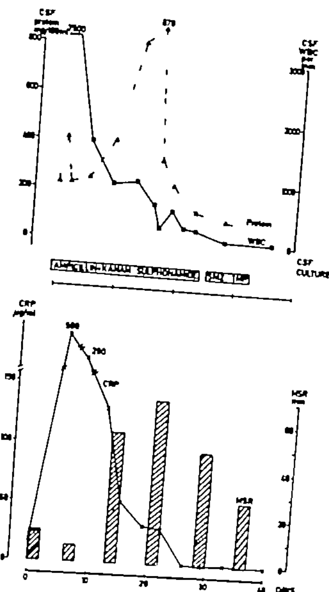


Fig 4 Case 2 See caption to Fig 3

Her development was normal when last seen at 7 months of age with no signs of sequelae

CRP in this case A high value of 86 µg/ml was seen even at 16 hours of age when the suspicion of infection was roused (Fig. 3) A rapid increase to 200 µg/ml was seen on the next day During the first period of treatment CRP fell but a plateau at a level of about 30 µg/ml remained when therapy was stopped When the relapse occurred a new peak to 200 µg/ml was seen. During the second set of therapy CRP rapidly fell but was still around 30 µg/ml as long as bacteria were present in the CSF A value below 10 µg/ml was obtained after one week and a zero value after another week on the 28th day of illness. Therapy was stopped 4 days after that when the CSF/blood glucose ratio was still below 0 5 No relapse occurred

Case 2

Boy born after 42 weeks gestation. He was asphyctic with an Apgar score of 3 at 1 minute and was resuscitated His mother had clinical signs of endometritis 2 days after delivery On the second day the boy had high tonus and was irritable A lumbar puncture showed bloody as well as purulent CSF with a low CSF/blood glucose ratio and Gram-negative bacteria were found (Fig 4) He was immediately started on ampicillin and kanamycin *E. coli* grew in cultures from CSF and blood and could still be isolated from CSF after 7 days on therapy The course of the illness was protracted, after 16 days the protein in CSF had reached 870 mg/100 ml, WBC were still above 500 per mm³ and now *Klebsiella* grew in cultures from CSF Some improvement was seen after sulfa-isodimidine had been added but at 3 weeks of age the boy was still listless had a grey colour and did not gain weight WBC count was 53 000 per mm³ MSR 69 mm and he had a progressive anemia to 6 7 g/100 ml Transillumination of the skull and subdural taps were negative

Therapy was changed to a combination of sulfamethoxazole and trimethoprim. The clinical condition improved rapidly The leucocytosis disappeared MSR fell and the protein and cell count in CSF became normal but the CSF/blood glucose ratio remained low He was dismissed from hospital at 6 weeks of age in good condition and when last seen at 14 months of age he was normally developed and showed no signs of sequelae

CRP in this case On the second day after birth, when cerebral irritation was first noticed a relatively low value of 18 µg/ml was found On the fifth day of illness a peak value of 500 µg/ml was reached (Fig. 4), the highest value of the whole series. CRP fell slowly and the high and broad peak was well correlated to the poor clinical condition the rising protein value the high cell count and the growth of *Klebsiella* in CSF After change of therapy the clinical condition rapidly improved CRP also fell rapidly from a plateau of 30 µg/ml to zero in 3 days Therapy was stopped 8 days later when the CSF/blood glucose ratio was still below 0 5 No relapse occurred

Case 3

Boy 6 weeks old who was admitted to the surgical ward because of a meteoristic abdomen and a tense hydrocele testis He was irritable with a temperature of 39–40°C His colour was pale he had increased tonus and absent Moro reflex A lumbar puncture and a blood culture was done but he was then already on ampicillin (day 1 Fig. 5). A meningitis was diagnosed with some 600 WBC per cmm in CSF protein of 160 mg/100 ml and a low CSF/blood glucose ratio of 0.27 Bacterial meningitis was suspected and he was put on penicillin G chloramphenicol and sulfa-isodimidine Cultures of CSF and blood were negative During the next few days the condition deteriorated He had frequent left-sided convulsions. EEG showed a focus of activity in the temporal and occipital region on the right side and subdural punctures showed a slight amount of purulent fluid from that side After 3 days he had attacks of apnea and had to be ventilated with a respirator for 48 hours and was given treatment for cerebral oedema. After that improvement was obvious, but on the 12th

AMYLASE CONTENT OF MIXED SALIVA IN CHILDREN

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ABSTRACT Rossiter M. A., Barrowman, J A., Dand, A. and Wharton, B. A. (Queen Elizabeth Hospital for Children, Hackney Road, London E.2 and the London Hospital, Whitechapel, London, E.1., England). Amylase content of mixed saliva in children. Acta Paediatr Scand, 63: 389 1974.—Salivary amylase levels were determined in normal subjects from birth until adult life and in children with conditions sometimes associated with low pancreatic amylase such as malnutrition, coeliac disease and cystic fibrosis. Mixed saliva was collected under carefully standardised conditions and amylase was measured by the method of Dahlqvist. There was wide scatter of values in the 84 normal subjects, but concentrations rose from very low levels at birth to reach adult levels by the age of 6 months to 1 year Salivary amylase activity rose normally over ten weeks in one premature infant fed milk by gastrostomy Thirteen children with coeliac disease and 9 children with cystic fibrosis mostly had normal salivary amylase concentrations. Six out of 12 malnourished children with jejunal villous atrophy of uncertain aetiology had low levels which rose to normal as recovery began.

KEY WORDS. Amylase, saliva, children, malnutrition

The amylolytic activity of mixed saliva is lower at birth than in childhood or adult life (10 14 16) and similarly pancreatic amylase is low in early infancy rising to adult levels by 2 years of age (6 9 10) The previous studies of amylase in saliva however have included few infants and have depended on less reliable biochemical methods than those available at present. Furthermore it is not known whether disorders affecting the pancreas in childhood also affect salivary amylase secretion The present study therefore was designed to determine the level of salivary amylase in normal subjects at birth and at different ages and in patients with conditions known to be associated with low pancreatic amylase such as malnutrition (2) coeliac disease (1) and cystic fibrosis (9).

METHODS

The subjects studied fell into the following groups:

Normal children [illegible] 4 term babies born at the Mother's Hospital, Clapton London, 27 infants and toddlers attending a well-baby clinic, 33 hospital patients (aged 1 month to 12 years) with conditions unrelated to the gastrointestinal or respiratory systems and 10 adult hospital workers. The infants studied had commenced starch-containing foods between the ages of 2 and 4 months.

Malnourished children. 12 patients 11 infants and one 5-year-old boy with prolonged malabsorption of uncertain aetiology Eleven had varying degrees of jejunal villous atrophy at the time of the first saliva sample in only one case was jejunal biopsy not performed. The nutritional status was assessed according to the Wellcome classification, i.e. weight below 60% of the Boston median for age: "severe malnutrition" weight 60–80% of the Boston median, "underweight" (12).

5 of the children were severely malnourished when first studied and 7 were underweight. None was oedematous

Coeliac disease 13 children (aged 7 months to 14 years) who at the time saliva was obtained, were receiving gluten and had sub-total villous atrophy of the jejunum. Eight had never been treated and have subsequently responded to a gluten-free diet, 1 had relapsed following discontinuation of a gluten-free diet 5 years previously and 4 previously-treated children were receiving gluten powder prior to diagnostic biopsy Four were "underweight"

Cystic fibrosis 9 children (aged 3 months to 11 years) of whom 3 were "underweight"

Gastrostomy A preterm baby (birth weight 1.9 kg.

cultures from blood and CSF In the cases with *H influenzae* CRP levels were closely related both to bacterial findings WBC and protein in CSF In meningitis caused by coli forms CRP was a more reliable parameter *than protein WBC in CSF* or *CSF/blood* glucose ratio or MSR and WBC in blood The finding that CRP was still increased in all cases where relapses later were found even if cultures at that time were negative and other parameters such as protein and WBC in CSF were getting normal is promising since the diagnosis of suboptimal therapy or other causes of relapses at present is diffi cult especially in the neonate (10) The course of the CRP levels in the 2 cases with purulent meningitis treated with ampicillin before cultures were obtained closely imitate those of *E coli* meningitis CRP can thus give a clue to the diagnosis and be of help in the treatment in such patients as illus trated by case 3

It has been shown *in vitro* that CRP in whole serum is more or less specifically bound to bacteria present in i a *pneumococci Staph aureus E coli* and *Klebsiella* species (6) and is probably changing the bacterial surface thus promoting phagocytosis (5) The effect seems to be dose-dependent (4) The presence of CRP as a phagocytosis-promoting factor thus seems to be an indirect sign of the pres ence of bacteria in blood or CSF This may explain why CRP is a useful guide for the course of illness and the efficiency of therapy in septicemia and meningitis of infants espe cially since other causes of increased CRP than bacterial infections seem to be rare in this age group Other tests of increased phagocytosis i e NBT-test is less reliable in infants espe cially in the first month of age (9)

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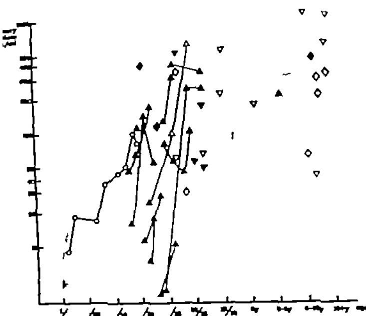


Fig 2 Salivary amylase concentrations in ill children compared with the normal range (shaded area) ▽=coeliac disease △=other enteropathy ◇=cystic fibrosis, ○=premature infant with gastrostomy The filling in of the symbols represents the degree of malnutrition, i.e. △=normal weight ▲="underweight" ▲="severely malnourished" Symbols joined by a line indicate results in the same subjects.

amylase at the same rate as normal term babies

DISCUSSION

Unstimulated whole saliva and stimulated parotid saliva show a circadian rhythm for flow and electrolyte composition (5) Flow rate is also influenced by mechanical factors such as jaw movements (11) In this study flow was stimulated chemically by lemon juice and probably mechanically by catheter suction. Specimens were obtained in the middle of the day when salivary secretion is maximal (5) Mixed saliva was studied because it can be obtained readily at all ages and because it is the overall amylolytic activity of saliva which is of practical importance to a child who is given starch-containing foods As mixed saliva is derived from several glands the results obtained in this study represent the amylolytic activity in this mixture collected under a carefully standardised set of conditions. For this reason our results can only be used to make comparisons within the groups studied that is comparison of different age groups and of healthy versus sick infants.

Normal subjects

There was great variation in the concentration of amylase in the saliva of normal subjects particularly from 6 months to 2 years but there was a steady rise from the very low levels at birth to reach adult values by the age of about one year so confirming the earlier work of Nicory (16) and Mayer (14) The rise in the concentration of amylase in mixed saliva could be due to a relative increase in the contribution made by the parotid gland the principal source of amylase However the observation of Kamaryt & Fintajslova (10) that the serum levels of salivary amylase rise during infancy suggests alternatively that there is an absolute increase in salivary amylase secretion presumably due to maturation.

Starch appears to induce pancreatic amylase production in newborn babies (17) and this may be one cause of the rise in salivary amylase concentration with age However since there was a 9-fold rise in the median salivary amylase concentration in the first month of life when no starch was given and since the premature baby fed milk for 10 weeks by gastrostomy developed salivary amylase at the normal rate for term babies, it seems that oral starch is not essential for development of the enzyme the parts played by intragastric nutrition and mechanical stimulation of the mouth in the

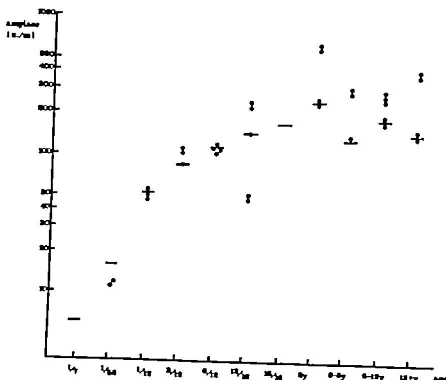


Fig 1 Salivary amylase concentrations in normal subjects showing the median value (—) for each age group

gestation 32 weeks) who following closure of a tracheo-oesophageal fistula at birth was fed milk by gastrostomy until repair of oesophageal atresia 10 weeks later weight gain during this time was 1 5 kg. Continuous suction was applied to the proximal oesophagus via a naso-oesophageal tube but no nutrients were put in the mouth until after the second operation

Collection of samples of saliva

Saliva was collected between feed between 10 00 and 16 00 hours Excess saliva was first aspirated from the buccal cavity then one drop of lemon juice was put on the tongue After 15 seconds, the saliva which had accumulated in the sublingual region was aspirated using a soft plastic catheter Specimens were deep-frozen until assay the longest period of storage being 4 weeks

Amylase activity was estimated by the method of Dahlqvist (4) As there was a large range of amylase activity in the different groups appropriate dilution was necessary the most active samples requiring 500-fold dilution.

RESULTS

Range in normal subjects

Fig 1 shows the concentrations of amylase in mixed saliva of normal subjects There is a wide scatter but the concentrations rose from very low levels at birth to adult values between 6 months and 1 year

Patients

Fig 2 shows the results in the patients with coeliac disease malnutrition due to other forms of enteropathy cystic fibrosis and tracheooesophageal fistula compared with the range found in normal children

Six of the 12 children with malnutrition initially had low salivary amylase concentrations the levels rose rapidly with treatment as the signs of malabsorption diminished but before normal weight was attained A further 2 infants in this group had salivary amylase levels within the normal range these children had already shown signs of recovery although they were still severely malnourished The remaining 4 children with severe malabsorption had normal salivary amylase levels

The only child with coeliac disease who had a low concentration of salivary amylase was a well-grown girl with asymptomatic enteropathy following 9 months challenge with gluten Four of the children with coeliac disease had levels in the low normal range

Two children with cystic fibrosis had low amylase levels and one a very high level There was no correlation with the state of nutrition

The pre-term infant with tracheo-oesophageal fistula who had had no oral feeding until the age of 10 weeks developed salivary

Acta Pædiatr Scand 63 393–397 1974

AN ENZYME INDUCTOR COMBINATION FOR THE PREVENTION OF HYPERBILIRUBINEMIA IN PREMATURE INFANTS

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ABSTRACT Hinkel, G. K., Kintzel, H.-W., Schwarze, R. and Händel, A. (Paediatric Hospital, Medical Academy "Carl Gustav Carus" Department of Neonatology Dresden, GDR). Enzyme inductor combination to prevent hyperbilirubinaemia in premature infants. Acta Paediatr Scand, 63: 393, 1974.—In the management of physiologic hyper bilirubinaemia in newborn infants, phenobarbital and nicethamide (diethylnicotinamide), as an enzyme inductor combination, are found to have a cumulative effect and, consequently are clearly superior to the use of phenobarbital alone. Four different dosages were compared and the most favourable variant proved to be phenobarbital, 10 mg/kg body weight pro die from day 1 to day 3 (after birth), combined with nicethamide, 100 mg/kg per diem from day 1 to day 4. Exchange transfusion was no longer necessary in 400 premature infants who had received this type of prophylactic combination and thus the routine use of this inductor regimen can now be recommended.

KEY WORDS: Newborn infants, hyperbilirubinaemia, enzyme inductor phenobarbital, nicethamide

Serum bilirubin levels in premature infants must be kept to a minimum if kernicterus is to be avoided. For prophylactic medication there are two alternatives—orotic acid (10 12 14 18 21 22, 29) or an enzyme inductor Over several years phenobarbital has proved to be an efficient inductor (23 26 27 28 30 31) However its suitability in premature infants is limited because of its respiratory-depressive action and because its effect upon serum bilirubin levels is slight.

The authors therefore have studied findings obtained from animal experiments 1 2, 3 5 9 24) and tested additional enzyme inductors known to be safely applicable in infants born at full term (8 25) No significant influence on serum bilirubin levels was recorded with nicotinamide phenylbutazone diazepam primidone and pyridion, while moderate effects were obtained by using nicethamide and meprobamate. Glutethimide was found to be even more effective than phenobarbital yet since exclusive use of phenobarbital or glutethimide was out of the question in the case of premature infants each substance was administered by the authors in conjunction with nicethamide (diethylnicotinamide) This was done with the intention that nicethamide an analeptic agent, should offset the respiratory-depressive action of the drugs and that such a combination might achieve an enhanced inductive effect. Simultaneous application of glutethimide and nicethamide failed to yield any effect beyond that obtained from glutethimide alone. Yet markedly additive induction was achieved in infants born at full term by combined application of phenobarbital and nicethamide (19) This effect was reproducible in premature infants (7 11). The same

gastrostomy fed infant are a matter for conjecture

Ill children

Six of the 12 malnourished children had initially low levels of salivary amylase levels rose rapidly with clinical improvement before a good weight gain was achieved Since all except one of the children with coeliac disease had normal levels of salivary amylase it seems probable that the low levels in the malnourished children were due to the state of malnutrition rather than the associated jejunal lesion It is not clear however whether malnutrition causes salivary amylase to fall or whether it prevents its development The older child who had severe malabsorption had a normal salivary amylase concentration We are unaware of salivary amylase studies in children with kwashiorkor

Although in cystic fibrosis the volume and bicarbonate concentrations of pancreatic juice are more conspicuously affected than the secretion of enzymes the majority of children with cystic fibrosis do have low concentrations of amylase in the duodenal juice (9 15) Since cystic fibrosis affects exocrine glands (7) a low concentration of amylase in saliva might have been expected but in this study most amylase concentrations were normal with only one conspicuously high and two low levels Chernick et al (3) found greatly raised levels of amylase and ribonuclease in the stimulated submaxillary saliva of 9 children with cystic fibrosis Di Sant Agnese et al (8) found normal levels of amylase in mixed and parotid saliva whereas Mandel et al (13) in a larger series of patients showed amylase concentrations to be raised in submaxillary but not parotid saliva

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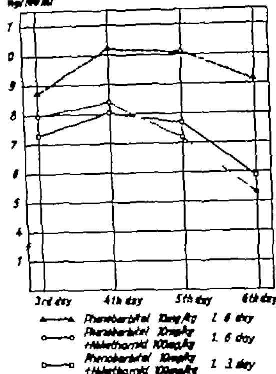


Fig. 1. Serum bilirubin levels in three groups of premature infants treated with phenobarbital and phenobarbital-nicethamid.

phenobarbital alone (Group I) when applied from the fourth to sixth day of age. Differences were significant on the fourth day with $p<5\%$ as well as on the fifth and sixth days with $p<1\%$.

2. No significantly different responses were recorded from bilirubin levels to 3, 4 or 6 days of combined treatment (Groups II, IV, V).

3. Short-term induction using phenobarbital plus nicethamide was accompanied by the highest bilirubin concentrations (Group III).

Behaviour of bilirubin maxima

Whereas exchange transfusions were unnecessary *in all four* combination groups described in this paper, the threshold of 18 mg/100 ml total bilirubin was exceeded in 3 of 76 premature infants who had received phenobarbital prophylaxis only (Table 3). The frequency of hyperbilirubinemia in excess of 15 mg/100 ml after short-term induction was higher than that observed after the three other variants of combined prophylactic treatment ($p<5\%$). After short-term induction, *the bilirubin maximum* was reached on the fifth day of age, but in Groups II, IV and V the maximum values were reached on the fourth day.

DISCUSSION

The superiority of phenobarbital with nicethamide as an inductor combination over the use of phenobarbital alone has previously been discussed by us (11, 19). Several variations were tried out with daily administration of 10 mg/kg phenobarbital plus 100 mg/kg nicethamide proving to be most favourable. The sedative action of phenobarbital was adequately offset by nicethamide when the above combination dosage was used over a short period of time. Nicethamide was not found to arouse irritability. Yet long term application was followed by an emerging dominance of barbiturate action attributable to phenobarbital accumulation.

Table 3. *Frequency of hyperbilirubinaemia by groups (total bilirubin)*

Group	Above 18 mg/100 ml		Above 15 mg/100 ml		No. of children
	Absolute	Per cent	Absolute	Per cent	
I	3	3.9	15	19.7	76
II	0	–	3	3.9	77
III	0		4	16.0	25
IV	0		3	3.7	81
V	0	–	1	1.3	76

Table 1 *Distribution of average birth weights among groups*

Group	Average birth weight (g)	No of children
I	1845	76
II	1935	77
III	1976	25
IV	1995	81
V	1970	76

enzyme inductor combination led the authors to almost a one third reduction in the number of exchange transfusions necessary to cope with Rh induced haemolytic disease of the newborn (20)

The studies described in this paper were undertaken with the object of establishing an optimum dosage for combined application of phenobarbital and nicethamide in premature infants so that the method may be introduced in routine practice

PATIENTS AND METHODS

The following approaches were taken alternatively to premature infants kept in one ward (small-for-date infants excluded)

Group I 10 mg/kg body weight phenobarbital *per diem* from day 1 to day 6 of age

Group II 10 mg/kg body weight phenobarbital *per diem* combined with 100 mg/kg nicethamide *per diem* from day 1 to day 6 of age (long-term induction)

Group III 10 mg/kg body weight phenobarbital plus 25 mg/kg nicethamide in 12th, 24th, and 36th hour of age (short-term induction)

Group IV 20 mg/kg body weight phenobarbital on first day of age 10 mg/kg on second and third day of age combined with 100 mg/kg nicethamide *per diem* from first to fourth day of age

Group V 10 mg/kg body weight phenobarbital *per diem* from first to third day of age combined with 100 mg/kg nicethamide *per diem* from first to fourth day of age (proposed for optimum dosage).

Both preparations were given orally with phenobarbital being given as a 2% solution in two single doses *per diem* and nicethamide as a 25% solution in six single doses *per diem* All five groups of infants were otherwise managed identically Birth weights were evenly distributed in the above groups. (Table 1)

RESULTS

Response of serum bilirubin levels

Serum bilirubin concentrations were compared after combined prophylactic application of phenobarbital with nicethamide and exhibited unambiguous dependence on both dosage and duration of treatment (Table 2 and Fig 1)

1 The bilirubin-depressive effect of phenobarbital combined with nicethamide (Groups II IV and V) was stronger than that of

Table 2 *Arithmetic mean values ($\bar{x}$) and standard deviations (s) of serum bilirubin concentrations from third to sixth day after birth for premature infants of different groups*

n = number of children *t* = total *i* = indirect

Group		Third day		Fourth day		Fifth day		Sixth day	
		t	*i*	*t*	*i*	*t*	*i*	*t*	*i*
I (*n* = 76)	$\bar{x}_1$	8 7	8 1	10 1	9 4	10 1	9 4	9 1	8 5
	s_1	2 8	2.5	3 6	3 7	2.9	3 0	3.3	3 2
II (*n* = 77)	$\bar{x}_2$	8 0	7 6	8 4	8 1	7 2	6.8	5.3	5 0
	s_2	2 9	2.8	3 4	3 3	3 3	3 3	2.9	2.8
III (*n* = 25)	$\bar{x}_3$	9 0	8 6	10 6	10 0	11 0	10.5	9 8	9 3
	s_3	2 2	2.2	2.8	2.7	3 5	3 4	3 7	3 6
IV (*n* = 81)	$\bar{x}_4$	7 1	6.8	8 1	7 6	7.3	6.8	6.1	5.5
	s_4	2.2	2.5	2.7	2 7	2.9	2.9	3 4	3 4
V (*n* = 76)	$\bar{x}_5$	7.3	6.9	8 0	7 6	7 7	7 1	5 9	5.2
	s_5	3 6	3 7	8.2	7.8	10 7	9 9	9.8	9 5

Total and non-conjugated serum bilirubin was determined by the method of Jendrassik (15 16) from third to sixth day after birth

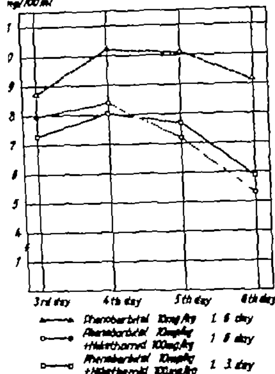


Fig 1 Serum bilirubin levels in three groups of premature infants treated with phenobarbital and phenobarbital-nicethamid.

phenobarbital alone (Group I) when applied from the fourth to sixth day of age. Differences were significant on the fourth day with $p<5\%$ as well as on the fifth and sixth days, with $p<1\%$

2. No significantly different responses were recorded from bilirubin levels to 3 4 or 6 days of combined treatment (Groups II IV V)

3 Short-term induction using phenobarbital plus nicethamide was accompanied by the highest bilirubin concentrations (Group III)

Behaviour of bilirubin maxima

Whereas exchange transfusions were unnecessary *in all four combination groups* described in this paper the threshold of 18 mg/100 ml total bilirubin was exceeded in 3 of 76 premature infants who had received phenobarbital prophylaxis only (Table 3) The *frequency of hyperbilirubinemia in excess of* 15 mg/100 ml after short-term induction was higher than that observed after the three other variants of combined prophylactic treatment ($p<5\%$) After short-term induction *the bilirubin maximum was reached on* the fifth day of age but in Groups II IV and V the maximum values were reached on the fourth day

DISCUSSION

The superiority of phenobarbital with nicethamide as an inductor combination over the use of phenobarbital alone has previously been discussed by us (11 19) Several variations were tried out with daily administration of 10 mg/kg phenobarbital plus 100 mg/kg nicethamide proving to be most favourable The sedative action of phenobarbital was adequately offset by nicethamide when the above combination dosage was used over a short period of time Nicethamide was not found to arouse irritability Yet long term application was followed by an emerging dominance of barbiturate action attributable to phenobarbital accumulation.

Table 3 *Frequency of hyperbilirubinaemia by groups (total bilirubin)*

Group	Above 18 mg/100 ml		Above 15 mg/100 ml		No of children
	Absolute	Per cent	Absolute	Per cent	
I	3	3 9	15	19 7	76
II	0	–	3	3 9	77
III	0		4	16 0	25
IV	0	–	3	3.7	81
V	0	–	1	1.3	76

Table 1 *Distribution of average birth weights among groups*

Group	Average birth weight (g)	No of children
I	1845	76
II	1935	77
III	1926	25
IV	1995	81
V	1970	76

enzyme inductor combination led the authors to almost a one-third reduction in the number of exchange transfusions necessary to cope with Rh-induced haemolytic disease of the newborn (20)

The studies described in this paper were undertaken with the object of establishing an optimum dosage for combined application of phenobarbital and nicethamide in premature infants so that the method may be introduced in routine practice

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Group III 10 mg/kg body weight phenobarbital plus 25 mg/kg nicethamide in 12th, 24th, and 36th hour of age (short-term induction)

Group *IV* 20 mg/kg body weight phenobarbital on first day of age 10 mg/kg on second and third day of age combined with 100 mg/kg nicethamide *per diem* from first to fourth day of age

Group V 10 mg/kg body weight phenobarbital *per diem* from first to third day of age combined with 100 mg/kg nicethamide *per diem* from first to fourth day of age (proposed for optimum dosage).

Both preparations were given orally with phenobarbital being given as a 2% solution in two single doses *per diem* and nicethamide as a 25% solution in six single doses *per diem* All five groups of infants were otherwise managed identically Birth weights were evenly distributed in the above groups (Table I)

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n = number of children *t* = total *i* = indirect

Group	Third day *t*	Third day *i*	Fourth day *t*	Fourth day *i*	Fifth day *t*	Fifth day *i*	Sixth day *t*	Sixth day *i*
I (*n* = 76) $\bar{x}_1$	8 7	8 1	10 1	9 4	10 1	9 4	9 1	8 5
s_1	2 8	2.5	3 6	3 7	2 9	3 0	3 3	3 2
II (*n* = 77) $\bar{x}_2$	8 0	7 6	8 4	8 1	7 2	6.8	5 3	5 0
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s_5	3 6	3 7	8.2	7 8	10 7	9 9	9.8	9 5

Total and non-conjugated serum bilirubin was determined by the method of Jendrassik (15 16) from third to sixth day after birth

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Consequently the authors became interested in minimizing application time for the inductor combination but without reduction in enzyme induction effect Our results are appear to suggest that the short-term induction applied to Group III was insufficient since the serum bilirubin values in that Group were higher than those recorded after administration of phenobarbital alone This series therefore was stopped before the scheduled deadline

Group IV received the inductor combination for 3 days with the phenobarbital dose being increased on the first day of age Although the serum bilirubin values were in agreement with those obtained by long term induction (Group II) this approach cannot be recommended for routine because of the marked barbiturate effect The dosage used in Group V was well tolerated Nicethamide was given for one day after barbiturate administration had been stopped taking into account that the degradation rate of phenobarbital in the organism is considerably slower than that of nicethamide Nicethamide also had to be administered six times a day because of its short half life The average serum bilirubin values in Group V and the frequency of bilirubin maxima in excess of 15 mg/100 ml were in full agreement with the results of long-term induction (Group II)

For the time being an enzyme inductor combination using phenobarbital with nicethamide is considered the most promising approach to prophylactic medication to prevent hyperbilirubinemia in newborns both premature and those born at full term The comparative studies described by the authors indicate that for maximum induction effect it will be sufficient to apply the dosage combination given in Group V to premature infants over a period of 3 days It is quite obvious that the impact of such an approach upon serum bilirubin levels will be clearly superior to the effect obtainable from orotic acid (11)

So far 400 premature infants to whom phenobarbital combined with nicethamide was therapeutically administered are under the authors care none of whom have required exchange transfusion Serum bilirubin values between 15 and 18 mg/100 ml were recorded in less than 4% of all cases provided that dosages were chosen properly The highest observed total bilirubin value was 16 8 mg/100 ml

The pharmacological properties of phenobarbital and nicethamide the two substances chosen for induction have been known for a long time Antagonistic action of phenobarbital against nicethamide and vice versa was offset on a recipropal basis in the intended way by the dosage used for Group V There is no evidence that either nicethamide (4 5 17) or phenobarbital (4 17) will free bilirubin linked to albumin Prophylactic routine use of the enzyme inductor combination phenobarbital/nicethamide in the manner used in Group V is therefore recommended as a preferred method to prevent the development of hyperbilirubinemia in premature infants On the other hand we consider phototherapy to be the most suitable method to treat a pre-existing hyperbilirubinemia. However the question of potential side effects of this treatment are not yet clear

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Table 1 *Clinical data Individual and mean values with S.D from 11 shunt operated patients with Fallot's tetralogy*

Pat.	Sex	Age (years) at Invest.	Age (years) at Shuntop.	B.S.A. (m^2)	P_{aO_2} (mmHg)	P_{aCO_2} (mmHg)	Max O_2-uptake 1 × (min^{-1} STPD)	Blood pres aort aorta (mmHg)	Hb g/100 ml	Hematocrit (%)
H. A.	M	33	13	1.59	57	38	1.62	120/73	19.2	60
K. A.	F	23	5	1 18	60	38	0.51	105/60	22.1	68
E. C.	M	22	4	1 70	57	–	1 05	105/–*	24.2	77
L. E.	M	33	[illegible]	1 54	61	31	1 45	119/75	21.6	70
S. E.	F	30	11	1.58	–	32	1 02	105/–	13.9	44
S. G	F	35	12	1.82	–	31	1 01	108/–*	12.2	43
B. J	F	26	8	1 77	68	30	0 92	100/63	15 1	51
A L.	M	25	7	1 45	62	29	0 76	134/–*	12.8	42
M. N	F	28	7	1 74	58	30	1.03	95/55	18.9	59
L. S.	F	32	10	1.68	80	31	0 97	103/54	12.6	38
A.-K S.	F	27	4	1.60	–	–	0 67	–	4.8	82
Mean				1.60	62.9	32.2	1 00	109.8	17.9	57.6
S D.				0.18	7.8	3 4	0.32	63.6	4.8	15.2

* Systolic pressure in right ventricle

of sodium balance and renal regulations of acid-base balance. All renal functional studies were performed during water diuresis. For this purpose the patients ingested water in an amount corresponding to 0.5% of the body weight every 30 minutes. The study was generally started 2-3 hours after initiation of the high fluid intake.

The glomerular filtration rate and the clearance of PAH were determined by a single injection technique (25 34) A solution containing 9% of inulin (Laevosan Gesellschaft) and 18% of para-amino hippuric acid (PAH) was given intravenously in the amount of 0.75 ml/kg body weight. Blood samples were taken at 5 minute intervals during the first 20 minutes after the injection and at 10 minute intervals during the next 60 minutes. From the plasma disappearance curve then obtained, the clearances of inulin (C_{in}) and PAH (C_{PAH}) were calculated from the formula given by Sapirstein (25) The filtration fraction (FF%) was calculated as $(C_{in}/C_{PAH}) \times 100$

To examine the renal regulation of sodium balance the urinary excretion of an oral salt load was studied The test has previously been carried out and evaluated in healthy subjects in this laboratory (6). In order to obtain as standardized conditions as possible the patients were kept on daily sodium intake of 100 mEq/1 73 m^2 body surface/day on the two days preceding the test. The urine samples were collected by spontaneous voiding at hourly intervals, the first of them 2 3 hours after starting of the high fluid intake. During the first 15 minutes of the second collection period the oral salt load was given as sodium chloride tablets (ACO), 95 mEq/1 73 m^2 body surface area After the administration of sodium chloride another 5 or 6 urine samples were collected.

The regulation of acid base balance was examined by studying the effect of an oral load of ammonium chloride (15 5) After control sampling of blood and urine ammonium chloride in a dose of 150 mEq/m^2 body surface area was given. Five hourly urine samples were then collected. Blood samples for determination of pH bicarbonate and P_{CO_2} were obtained in the middle of each urine collection period. The results of the ammonium chloride test were interpreted from the relationship between the urine pH and the concentration of total blood CO_2. In healthy children this relationship has been found to be characteristic (5).

In addition determinations were also made of the working capacity (4). The arterial blood pressure was generally recorded with a cuff or directly during cardiac catheterization. The P_{O_2} and the hematocrit of arterial blood was determined. The daily urinary aldosterone excretion was determined in 4 patients. Routine urine analysis and urine culture were carried out in all patients and were consistently normal.

Analytical methods

The concentration of inulin in the blood was determined by the anthrone method (18) and the concentrations of PAH by the method of Smith (29).

The sodium and potassium concentration in serum and urine was determined by flame photometry

The pH in blood and urine was determined with a pH meter (Radiometer). Standard bicarbonate was determined by analysing the pH after equilibrating blood samples with 4 and 8 per cent of carbon dioxide. By placing the pH results in a Siggard-Andersen curve nomogram and correcting for the low arterial P_{O_2}, the P_{CO_2} and actual bicarbonate concentration were obtained (28). The arterial P_{O_2} was determined with a Radiometer electrode The hematocrit of heparinized blood samples were determined by centrifuging them at 10000 rpm for 5 minutes. Aldosterone in urine was determined with a double isotope method (20). For statistical analysis the Student's t-test has been used.

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RENAL FUNCTION IN FALLOT'S TETRALOGY

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ABSTRACT Aperia A Bjarke B Broberger O and Thorén, C (Department of Paediatrics, Karolinska Institutet S.t Göran's Children's Hospital, Stockholm, Sweden). Renal Function in Fallot s Tetralogy Acta Paediatr Scand, 63: 398, 1974.—Renal function was examined in 11 adults with Fallot's tetralogy The following aspects of renal function were studied. GFR PAH clearance filtration fractions, the natriuretic response to an oral salt load and renal regulation of acid base balance The GFR was found to be only moderately reduced while PAH clearance showed an almost 50% reduction in most cases studied. Consequently the filtration fraction was abnormally high The natriuretic response to an oral salt load was generally low The urinary sodium elimination rate was found to be independent of deviations in GFR and correlated inversely with the filtration fraction. The renal response to ammonium chloride induced acidosis was studied in 7 patients and was delayed in at least 3 of those The pathological response was thought to be due to hypocapnic depression of renal HCO_3 reabsorption It is suggested that this could contribute to the mild nonlactic acidosis often present in congenital cyanotic heart disease

KEY WORDS Tetralogy of Fallot glomerular filtration rate filtration fraction sodium balance acid base balance

The kidney is a unique organ in that the blood supply will to a large extent determine the work load i e the glomerular filtration rate (33) One might therefore suspect that when the general circulatory pattern is disturbed such as in congenital heart disease renal function will also be affected

The circulatory changes in heart disease might however also be expected to increase the demand on the kidney as a homeostatic organ A study has therefore been carried out to evaluate the homeostatic function of the kidney in congenital heart disease The control of sodium balance in patients with aortic coarctation has recently been reported (3) The present report deals with patients with tetralogy of Fallot Of particular interest is the occurrence of polycythemia since experimental increase of the hematocrit has been reported to influence renal hemodynamics as well as renal sodium excretion (31 23 10 26)

This work was supported by grants B73-19X 2049-07A from the Swedish Medical Research Council and the Swedish National Assoc against Heart and Chest Diseases

MATERIAL

Eleven patients 3 males and 8 females were studied. The diagnosis Fallot s tetralogy was in all cases based on the findings from cardiac catheterizations and angiocardiography All the patients had been subjected to palliative shunt operation according to Blalock Taussig and had various degree of hypoxemia and polyglobulia. None of the patients had histories of urinary tract infections or other renal disease. Pertinent information about the patients including certain laboratory data is given in Table 1

METHODS

The patients were hospitalized for 5 days The following aspects of the renal function were examined: Glomerular filtration rate and PAH clearance renal regulations

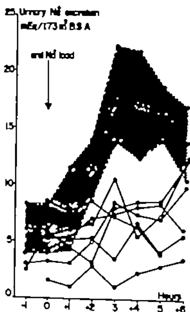


Fig. 2. Hourly sodium excretion (mEq/1.73 m² B.S.A.) following an oral sodium load of 95 mEq/1.73 m² B.S.A. in 7 patients. The outer limits of the shaded area represents the ranges of response found in normal children. The broken line within the shaded area represents the mean normal response.

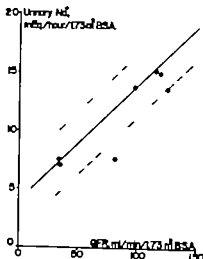


Fig. 3. The relationship between the glomerular filtration rate and the average hourly urinary sodium excretion 2–6 hours after the oral salt load. The solid line represents the line of regression, the broken lines ±2 standard deviations for 17 children with renal disease (filled circles) in whom urinary sodium elimination rate was found to correlate significantly with glomerular filtration rate (3). The values for 6 of the patients with Fallot's tetralogy are represented by the open circles.

to recurrent urinary tract infections the natriuretic response to the salt load is depressed as a function of the glomerular filtration rate (1). In the patients with Fallot however the natriuretic response is depressed out of proportion to the GFR (Fig. 3). Thus enhanced tubular reabsorption of sodium must contribute to the depressed natriuretic response. One of the factors known to enhance tubular sodium reabsorption is a high filtration fraction (26). The relationship between the average hourly urinary Na excretion 2–6 hours after the load and the filtration fraction in 6 of the patients is illustrated in Fig. 4. A significant inverse correlation was found when the mean hourly excretion of sodium was plotted against the filtration fraction ($r = 0.9355$, $p < 0.01$).

Aldosteron was quantitatively estimated in only 4 of the patients and found to be normal. Potassium/sodium ratios were however determined in 24 hours urine specimens from all 7 patients studied and did in no case exceed 0.51 strongly suggesting that no increase in aldosteron secretion was present (Table 3).

The renal regulation of acid base balance was examined in 7 of the patients. A detailed description of the interpretation of the short time ammonium chloride test has been given previously (5). When the curve is displaced to the left urinary bicarbonate loss is the main cause of a defect in acidification. On the

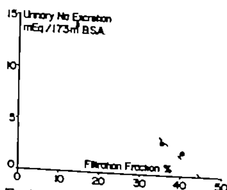


Fig. 4. Relationship between mean hourly urinary sodium excretion after an oral salt load (95 mEq/1.73 m² B.S.A.) and filtration fraction.

Table 2 *Renal functional data with glomerular filtration rate (GFR) PAH clearance (CPAH) and filtration fraction in Fallot s tetralogy with individual and mean values*

Patient	GFR ml/1 73 m² (B.S A./min)	CPAH ml/1 73 m² (B S A./min)	Filtration fraction (%)
H A	88 1	316.3	36
K A	71 0	231 0	30 6
E. C	109.2	245 0	44 6
L. E.	126 7	315 1	40.2
S E.	113 4	415.2	27.3
B J	106 3	361 0	29 4
A L.	81.2	402.7	20 2
M N	102.7	288.3	35 6
L. S	74 8	390 8	19 1
A K S	85 7	177 2	40 1
Mean	95 91	314 26	32.31
S D	18 36	79 75	8.54
S E.	5 80	25 22	2.70
p	<0 001	<0 001	<0.01
Normal values for the lab Mean ±S D	122.5±16.4	546 0±47.2	21 2±1.3

Six healthy children aged 8–14 years

RESULTS

All patients had a low P_{O_2} and with few ex ceptions a low P_{CO_2} (Table 1) The working capacity measured as (V_{O_2} max) was low and in most cases reduced to 50% or more of nor mal As could be expected hematocrit values were in general abnormally high and inver sely correlated to the degree of arterial de saturation

The values of glomerular filtration rate (GFR) PAH clearance and filtration fraction (FF) are given in Table 2 In 5 of the 10 pa tients in whom the GFR was examined the values were moderately reduced The clear ance of PAH was generally reduced but to a greater extent than the GFR yielding an ab- normally high filtration fraction

In Fig. 1 the filtration fraction (FF) is plotted against hematocrit (hct) There is a very close direct relationship between these two parameters

The renal control of sodium balance was examined in 7 of the patients by determining the natriuretic response to an oral salt load (Fig. 2) The results have been compared with the natriuretic response to a correspond ing load in healthy children aged 8–14 years

In normal subjects the hourly sodium ex cretion is high 2–6 hours after the load and as a rule exceeds 16 mEq/1 73 m² The natriuretic response was much lower in the patients with Fallot s tetralogy In some cases there was an almost complete lack of response

The inability to increase the urinary sodium excretion following a salt load could depend either on a reduction of the filtered load of sodium or an enhanced tubular reabsorption of sodium In patients with reduced GFR due

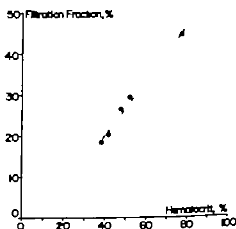


Fig 1 Relationship between filtration fraction (%) and hematocrit (%) in 10 of the patients.

dynamics in Fallot's tetralogy. Enlarged hypercellular and congested glomeruli with dilated afferent arterioles have been described in certain cases of congenital cyanotic heart disease (30). So far the functional significance of these glomerular changes is unknown.

The natriuretic response to an oral salt load was found to be low in patients with Fallot's tetralogy. The low urinary sodium excretion was not correlated to deviations in glomerular filtration rate and must therefore be attributed to an enhanced tubular reabsorption of sodium and to a reset of glomerular tubular sodium balance. Intrarenal physical forces including hydrostatic and oncotic pressure gradients between the peritubular capillaries and the peritubular interstitium are generally believed to be of primary importance for the control of tubular sodium reabsorption. Low hydrostatic (2, 22) and high oncotic pressure (8, 19) in the peritubular capillaries are believed to enhance the reabsorption of sodium. The renal artery perfusion pressure was most likely not markedly low in the patients and was therefore only of minor importance for the low elimination rate of sodium. On the other hand there is good reason to assume that the oncotic pressure in the peritubular capillaries is increased in Fallot's tetralogy since the filtration fraction is high, leading to a concentration of the plasma proteins in the postglomerular vessels. In the present study the values for urinary sodium excretion were found to correlate inversely with the filtration fraction. This will strongly suggest that the diminished urinary sodium excretion found in the patients is mainly attributed to changes in intrarenal physical forces mediated by the high hematocrit. A sodium retaining effect of high hematocrit has previously been found in dogs (10). Among other factors known to influence tubular sodium reabsorption only aldosterone was evaluated. As aldosterone levels as well as urinary potassium-sodium quotients were found to be normal in the present study, hyperaldosteronism can be eliminated as a cause of the low natriuretic response. The tendency to sodium retention does not seem to be of disadvantage for patients with Fallot's tetralogy. A positive sodium balance is known to predispose to renal hypertension (12) but so far there are no reports on hypertension in Fallot's tetralogy. Rather the patients may benefit from sodium retention by increasing their plasma volumes and thereby preventing the hematocrit to reach too high levels.

Low arterial P_{CO_2} values due to hyperventilation was a common finding in patients with Fallot's tetralogy. A fall in arterial P_{CO_2} is known to depress the renal HCO_3-reabsorption (24). It is therefore not surprising that signs of renal HCO_3-loss was found in at least 3 of 7 patients. Of interest is that mild nonlactic acidosis appears to be a consistent finding in patients with congenital cyanotic heart disease (32). It is quite possible that the hypokapnic depression of the renal HCO_3 reabsorption contributes to the acidotic tendency in cyanotic heart disease.

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Table 3 *Individual and mean values for serum concentration of sodium and potassium urinary potassium/sodium quotient*

Patient	Na mEq/l (serum)	K mEq/l (serum)	K/Na (urine)
H A.	140	4 6	0 47
K A	146	3.5	-
E. C.	145	5 0	-
L. E.	142	4 6	0 47
S E.	147	4 0	-
S G	143	4 4	0 48
B J	138	4 1	0.45
A L.	132	3 9	0 34
M N	141	4 4	0.51
L. S	147	4 0	0 23
A. K S	130	4 3	-
Mean	140.5	4 25	0 41
S D	5 4	0 41	0.10

other hand when there is a complete inability to depress the urinary pH below 5.3 bicarbonate reabsorption may be intact but the excretion of ammonia and titrable acids is defective In Fig. 5 the curves were interpreted as normal or border line normal for 4 of the patients with Fallot s tetralogy In the remaining 3 patients the curves were definitely displaced to the left indicating reduced bicarbonate reabsorption and increased urinary bicarbonate losses The fact that all patients were able to acidify the urine below pH 5 2 when the total CO_2 of blood was depressed enough makes a defect in urinary excretion of ammonium and/or titrable acids unlikely

The blood P_{CO_2} was consistently low but did not change characteristically during the induction of the acidosis In the 3 patients with pathological response AL SE and SG it averaged 28 0 29 8 and 28 0 mmHg respectively In the remaining 4 patients the average blood P_{CO_2} during the ammonium chloride load ranged between 28 8 and 37 3 mmHg

DISCUSSION

In order to evaluate the renal function in Fallot s tetralogy it will be necessary to define the circulatory and respiratory deviations which might influence the homeostatic mechanisms of the kidney Arterial desaturation seems to be the principal denominator of these factors among which can be recorded. polycythemia yielding a high hematocrit (7) and hyperventilation resulting in a low P_{CO_2} in the resting state (11 13) The high hematocrit seems to be the primary adaptive mechanism to the hypoxia The cardiac output does not seem to be increased (7 9). Low arterial P_{O_2} is at least in the acute state known to result in renal vasoconstriction (16, 17 21) In the present study the total renal blood flow was not estimated but both the glomerular filtration rate and the effective renal plasma flow (the clearance of PAH) were found to be reduced The reduction in glomerular filtration rate was only moderate while effective renal plasma flow was reduced to about 50% of normal yielding an abnormally high filtration fraction The low PAH clearance as well as the clearance of inulin is well explained by the high hematocrit as less plasma per unit blood will reach the kidney A high filtration fraction has previously been reported in patients with Fallot s tetralogy (27) as well as in animals with experimentally induced polycythemia (23 26 31) The results from the present study do not exclude that in addition some intrarenal factors will also effect renal hemo-

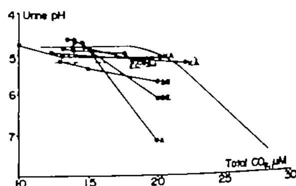


Fig 5 The relationship between blood total CO_2 and urine pH in 7 patients with Fallot s tetralogy The broken lines represent the outer limits of the values found in healthy children.

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KEY WORDS: Cystic fibrosis, diagnostic methods, nail clippings, neutron activation analysis

Cystic fibrosis of the pancreas (CF mucoviscidosis) is a generalized hereditary disease in which there is dysfunction of all or most exocrine glands (7) Its main clinical manifestations make a characteristic triad. Chronic pulmonary disease pancreatic deficiency and abnormally high concentrations of electrolytes in the sweat.

Since the outcome for patients with CF is highly dependent upon early diagnosis and treatment (9 17 19) there is a pressing need for a reliable diagnostic method preferably one which could also be used as a screening test in the neonatal period

Many authors (1 2, 4 8 11–16 18 20) have shown that abnormally high concentrations of electrolytes are present not only in the sweat but also in the nails of CF patients. Determination of the sodium concentration of nails has therefore been suggested as a diagnostic tool for CF by most of these authors but measurement of chlorine (21) and copper (12) have also been proposed

In the present study we have used instrumental neutron activation analysis (INAA) for the determination of sodium in the nails The aim of the study was (*a*) to find out different sources of error in the differentiation of patients from normal subjects and (*b*) to investigate to what extent nail sodium was correlated to sweat sodium.

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In the present study we have used instrumental neutron activation analysis (INAA) for the determination of sodium in the nails. The aim of the study was (*a*) to find out different sources of error in the differentiation of patients from normal subjects and (*b*) to investigate to what extent nail sodium was correlated to sweat sodium.

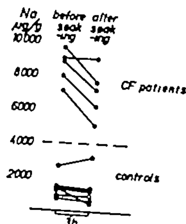


Fig 1 Sodium concentration of nail clippings from five CF patients and six controls before and three hours after soaking. In all figures ● represent patients with CF and O controls

MATERIAL AND METHODS

The free edges of finger nails were clipped from CF patients and controls all of whom were more than three years old (In the following "nails" or "nail clippings" always mean the free edges of the nails) In CF patients the diagnosis had been established unequivocally by double determinations of sweat electrolytes by pilocarpine-iontophoresis (3) in addition to the well-known clinical manifestations. Healthy boys and girls served as controls for the investigations of different sources of error whereas patients suffering from lung diseases other than CF were used as controls for studying the correlation between sweat sodium and nail sodium concentration

Unless otherwise stated and in accordance with experiences of Woodruff et al (21) the children had no periods of swimming in the week and no bath in the 24 hours preceding the clipping procedure After mechanical removal of visible dirt the entire nail was soaked with distilled water by means of a gauze pad. Three hours later the nails were clipped with scissors which had previously been washed in distilled water and put into HCl-cleaned plastic irradiation ampoules

Together with comparison standards containing known amounts of sodium the ampoules containing the nail clippings were placed into an irradiation can and irradiated for about 10 hours in a neutron flux of 6×10^{11}n/cm² sec The nail clippings were then transferred to inactive cans and the activity of ^{24}Na (from the reaction ^{23}Na (n γ)^{24}Na) was measured in nails and standards with conventional gamma ray spectrometry using a well type 3 ×3 Na(I) detector The concentration of sodium in the nails was obtained by comparing its specific activity with that of the standard

The accuracy and precision of the activation analyses were checked by analysis of the sodium concentration of the international biological reference sample of kale powder (5) which was irradiated from time to time together with the nail clippings Six analyses gave a mean sodium value of 2200 µg/g with a standard deviation of 110 µg/g.

OBSERVATIONS AND RESULTS

Investigations of different sources of error

Influence of the soaking procedure Nails from five CF patients and six controls were clipped from one hand three hours after soaking and from the other hand without soaking The soaking lowered the sodium concentration in the nails from both CF patients and controls except in two cases (Fig. 1). In no case however did the sodium in CF nails decrease to the concentration found in the control nails (lowest CF value 5080 µg/g, highest control value 3150 µg/g)

In one CF patient and one control child clippings were collected immediately before and at different intervals after soaking the last sample was taken after five hours (Fig. 2) The difference between the unsoaked nail of the CF patient (10700 µg/g) and the unsoaked control nail (756 µg/g) was considerable After the soaking the sodium concentration of the CF nails decreased to about half of the initial value and remained at that level fairly constantly for five hours whereas the concentration of the control nails stayed at about the same level as the unsoaked control nail throughout the five hours of observation

The effect of a one-hour bath Edges of all ten fingernails were clipped from one CF patient and from one control The first nail was clipped before a one-hour bath and the others were clipped 0–72 hours after the

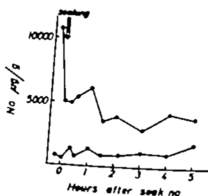


Fig 2 Sodium concentration of nail clippings before and at different intervals after soaking.

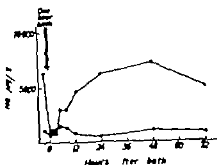


Fig. 3. Sodium concentrations of nail clippings before and after a one-hour bath.

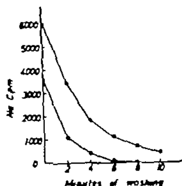


Fig. 4. Decrease of ^{24}Na activity in nail clippings during in vitro washing with distilled water.

bath (Fig. 3). A drastic drop of the sodium concentration in the nails of the CF patient *down to the level* of the control nails was observed immediately after the bath and the prebath values were not regained before 12–24 hours. The sodium concentration of the control nails was more stable.

Washing after irradiation (Fig. 4). During a 10-minute washing period in distilled water the residual activity of ^{24}Na in the nail clippings was measured at 2-minute intervals. An approximately exponential decrease of the activity was observed during this period, the residual activity of ^{24}Na being 1/10 or less of the initial value after 10 minutes.

Contamination studies. Dipping the nails for 30 minutes in dry crystal salt, physiologic salt solution (0.9% NaCl) or a saltrich hexachlorophene soap did not increase the sodium concentration of nail clippings from a healthy control to pathological values. Only contamination with highly concentrated solutions—2.8% NaCl (≈sea water) or more—gave concentrations up in the CF range.

Homogeneity. The free edge of the nail from a CF patient showed high amounts of sodium (21600 μg/g), whereas the fixed portion whether soaked or unsoaked contained sodium in the normal range (2440 μg/g and 2750 μg/g respectively). Furthermore, the sodium was not homogeneously distributed along the free edge. Considerable differences between the ulnar and radial region and from nail to nail of individual clippings were found both in one control and one CF patient (see Table 1). This variation was totally unsystematic. An overlap between controls and CF values never occurred, however.

Relation between sodium content in sweat and in nail clippings

In 12 CF patients and five patients with other lung diseases the sodium in nail clippings and

Table 1. *Sodium concentration of different parts of the free nail edges*
R = radial, U = ulnar. Values in μg/g.

	I		II		III		IV		V		
	R	U	R	U	R	U	R	U	R	U	Mean
Control											
Right	575	612	504	456	676	660	645	615	722	602	621
Left	810	445	870	730	630	680	604	520	473	571	
CF											
Right	4620	3970	4620	4440	4480	3530	5460	6480	10690	5930	5713
Left	4440	4020	8260	7830	4330	4150	5540	5330	9270	7620	

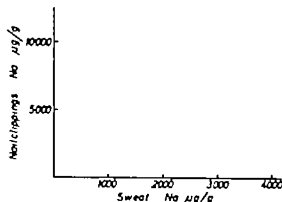


Fig 5 Comparison of sodium concentrations of nail clippings (analyzed by INAA) and sweat (analyzed by flame photometry)

the sodium in sweat after pilocarpine iontophoresis were simultaneously measured As seen in Fig 5 there was good correlation between these two parameters (correlation coefficient 0 71) CF patients had higher concentrations of sodium than controls both in the nails and in sweat

DISCUSSION

The different sources of error in the identification of CF by analysis of sodium in nail clippings may arise from the collection routine from the analytic procedure or from the biological material itself

To understand the errors which may result from the collection routine it is important to have a clear picture of the origin of the sodium in the nail Theoretically it may be divided into three parts according to its source the *intrinsic* part built into the nailplate either from the matrix or from the hyponychium the *extrinsic* part from other autobiological sources such as sweat and the *contaminative* part from environmental sources such as table salt soap salt solutions etc From this theory the question to solve was whether the elevated sodium concentration in the nails of patients with CF came from the intrinsic or the extrinsic part or both

The sodium concentrations in nail clippings from both CF patients and controls have varied in reports from different investigators This might be due to differences in the washing procedures In our study seven patients with CF had higher sodium concentrations in nails that were clipped without any soaking or bathing than in 19 out of 20 nails that were clipped 0–12 hours after such procedures This agrees with earlier observations (14 18 20 21) that water easily washes out sodium from the nails of patients with CF The effect of washing on control nails is on the other hand rather small This might indicate that the extrinsic and contaminative sodium contents of control nails usually are of little importance

The residual concentration of sodium after a prolonged bath and the concentration of the fixed portion of a nail of a CF patient may mainly reflect the intrinsic part This concentration seems to be about the same in CF nails and control nails

The contamination studies showed that only highly concentrated salt solutions gave pathologically high sodium concentrations in the nails This agrees with earlier observations (11 13) and indicates that the danger of contamination leading to falsely pathological values is small

In an attempt to eliminate the risk of sodium contamination from the washing medium we also observed the effect of in vitro washing after irradiation The rapid removal of ^{24}Na in this study is in concordance with earlier findings (11 14) and might be partly explained by a Szilard-Chalmers effect according to which the radionuclides formed are often easily extractable in various solvents (6)

Errors due to the analytic procedure may arise from the contribution to the ^{24}Na activity from threshold reactions such as ^{24}Mg (n p)^{24}Na and ^{27}Al (n α)^{24}Na. Such errors are however negligible due to the well thermalized neutrons in the irradiation position and the low content of Mg and Al in nails (10)

From the results of test analyses of an international biological reference sample the

accuracy and precision of the INAA is considered satisfactory for the present purpose A mean sodium value of 2200 $\mu g/g$ was obtained This value can be compared with the values obtained at other laboratories in the international study (5) Since the reference samples were analyzed routinely together with the nails in a screening series the standard deviation obtained (5%) should be a realistic value for the precision of the sodium analyses

Errors from the biological material may be due to an inhomogeneous distribution of sodium in the nail clippings or to an impaired contribution to the extrinsic part from the sweat To avoid misinterpretations due to the inhomogeneous distribution which is shown in this work and has been observed by Heintz (13) it is important to collect the largest possible sample of the free edges of nails from all fingers This will give a more reliable mean of the sodium concentration of the nails

The high correlation coefficient for the concentration of sodium in the sweat and nails is in agreement with the view that sodium from sweat is absorbed and accumulated by the nails (11 14 21). Further support for this hypothesis is found in the ease with which sodium can be removed from the nails of CF patients and the relative rapidity with which it can be restored. Such findings are not made in nails of healthy controls

The correlation between the sodium concentration of sweat and that of nail clippings is dependant upon several factors e.g. the number of sweat glands their activity their contact with the nail the absorption capacity of the nail and the degree of evaporation.

The method for determination of sodium in nail clippings should be so designed that if possible no falsely low values are obtained Falsely high values are of less consequence as all high readings must always be checked by sweat tests.

On the basis of our findings the following collection routine is recommended

No swimming is allowed in the week, no bath in the 24 hours and no handwashing in the 12 hours preceding the clipping procedure —The long standing depression of the sodium concentration after a bath comprises a strong reason for this prerequisite

After mechanical removal of visible dirt, as large an amount as possible of the free edges of the nails should be clipped No soaking should be undertaken before clipping, as washing might lead to too low values giving falsely normal results for nails of persons with CF In fact, the most discriminating results have come from studies with little or no washing (2, 8 14) Of course nails that are visibly contaminated with blood or extensive dirt should be avoided as well as those from a frequently sucked finger It is important to collect the free edges of the nails since in CF patients these have a much higher sodium concentration than the fixed portion

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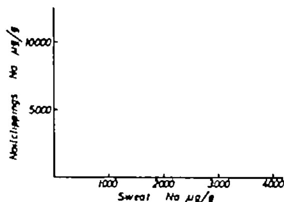


Fig 5 Comparison of sodium concentrations of nail clippings (analyzed by INAA) and sweat (analyzed by flame photometry)

the sodium in sweat after pilocarpine iontophoresis were simultaneously measured As seen in Fig 5 there was good correlation between these two parameters (correlation coefficient 0 71) CF patients had higher concentrations of sodium than controls both in the nails and in sweat

DISCUSSION

The different sources of error in the identification of CF by analysis of sodium in nail clippings may arise from the collection routine from the analytic procedure or from the biological material itself

To understand the errors which may result from the collection routine it is important to have a clear picture of the origin of the sodium in the nail Theoretically it may be divided into three parts according to its source the *intrinsic* part built into the nailplate either from the matrix or from the hyponychium the *extrinsic* part from other autobiological sources such as sweat and the *contaminative* part from environmental sources such as table salt soap salt solutions etc From this theory the question to solve was whether the elevated sodium concentration in the nails of patients with CF came from the intrinsic or the extrinsic part or both

The sodium concentrations in nail clippings from both CF patients and controls have varied in reports from different investigators This might be due to differences in the washing procedures In our study seven patients with CF had higher sodium concentrations in nails that were clipped without any soaking or bathing than in 19 out of 20 nails that were clipped 0–12 hours after such procedures This agrees with earlier observations (14 18 20 21) that water easily washes out sodium from the nails of patients with CF The effect of washing on control nails is on the other hand rather small This might indicate that the extrinsic and contaminative sodium contents of control nails usually are of little importance.

The residual concentration of sodium after a prolonged bath and the concentration of the fixed portion of a nail of a CF patient may mainly reflect the intrinsic part. This concentration seems to be about the same in CF nails and control nails.

The contamination studies showed that only highly concentrated salt solutions gave pathologically high sodium concentrations in the nails This agrees with earlier observations (11 13) and indicates that the danger of contamination leading to falsely pathological values is small

In an attempt to eliminate the risk of sodium contamination from the washing medium we also observed the effect of in vitro washing after irradiation The rapid removal of ^{24}Na in this study is in concordance with earlier findings (11 14) and might be partly explained by a Szilard-Chalmers effect according to which the radionuclides formed are often easily extractable in various solvents (6)

Errors due to the analytic procedure may arise from the contribution to the ^{24}Na activity from threshold reactions such as ^{24}Mg (n p)^{24}Na and ^{27}Al (n α)^{24}Na. Such errors are however negligible due to the well thermalized neutrons in the irradiation position and the low content of Mg and Al in nails (10)

From the results of test analyses of an international biological reference sample the

Acta Pædiatr Scand 63 411–417 1974

A CLINICAL STUDY OF THE DIAGNOSIS OF CYSTIC FIBROSIS BY INSTRUMENTAL NEUTRON ACTIVATION ANALYSIS OF SODIUM IN NAIL CLIPPINGS

H KOLLBERG and G EKBOHM

From the Department of Paediatrics University Hospital Uppsala and the Department of Statistics University of Uppsala Sweden

ABSTRACT Kollberg, H. and Ekbohm, G (Department of Paediatrics, University Hospital, Uppsala, Sweden and Department of Statistics, University of Uppsala Sweden). A clinical study of the diagnosis of cystic fibrosis by instrumental neutron activation analysis in nail clippings. Acta Paediatr Scand, 63: 411 1974.—This article reports on the possibility of using instrumental neutron activation analysis (INAA) of sodium in nail clippings for diagnosing cystic fibrosis (CF) in children and adults, for detecting heterozygotes and for screening in the neonatal period. Nail clippings from 1322 newborns, 22 CF patients (two of these newborns), 52 healthy controls and 22 heterozygotes were analysed. The discrimination between CF patients and controls was found to be precise for individuals above one year of age and INAA of nail clippings should be accepted as a diagnostic test for CF after this age. Heterozygotes could not be detected by the method. During the first five days of life there is a big overlap between the values from normal newborns and those of CF children, which makes the method invaluable for early screening for CF

KEY WORDS. Cystic fibrosis, nail clippings, neutron activation analysis, sodium, new borns

Cystic fibrosis of the pancreas (CF) a generalized disease with dysfunction of all or most exocrine glands is one of our most common hereditary disorders The incidence among Caucasians is generally reported to be 1 2000–4000 newborns (2 18). Early diagnosis and treatment offer the best possibility of improving the outcome for patients with this disease (5 13 15).

Compared with normal persons patients with CF have a higher concentration of sodium in their sweat (3) and nails (10) It has been considered that instrumental neutron activation analysis (INAA) of sodium in nail clippings might be a useful method for diagnosing CF (4 6, 8 9 14 17)

The aim of this study was to investigate the possibility of using INAA of sodium in nail clippings (*a*) for the diagnosis of CF in children and adults, (*b*) for the detection of heterozygotes of CF and (*c*) as a screening test for CF in the neonatal period

Supported by Grants from the Swedish Medical Research Council project Nos. B-9-19X 2582-01 and B-73-99Z 3281-03

MATERIAL AND METHODS

Nails were clipped from 28 CF patients, 22 heterozygotes of the disease and 52 healthy subjects. The CF patients and controls were defined according to the criteria of an earlier study (9). All the heterozygotes were parents (both fathers and mothers) of children among the CF patients. The material included nails from four CF children under one year of age. The ages of the other CF patients varied from one year to adulthood. The healty controls were mainly newborn—10 years old,

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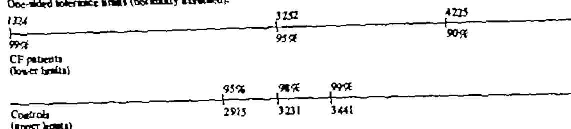
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Table 1 Comparison of the sodium concentration of nail clippings from CF patients heterozygotes and controls

Category	n	Mean	S.D	Range	99% confidence intervals for mean difference	
CF patients	28	7515	2460	3600–12800	6505±1264	5870±1228
Heterozygotes	22	1010	735	200– 3300	635± 487	
Controls	52	1645	765	290– 3600		



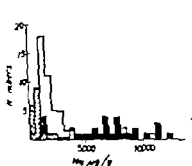




Fig. 1 Histograms for sodium concentration of nail clippings from CF patients, heterozygotes and controls. CF patients <1 year ☐, n=4 >1 year ■ n=24 heterozygotes ▣ n=22 controls ☐, n=52.

Fig. 2 Sodium concentration of nail clippings from CF patients and controls according to age ● CF patients, ○ controls × × mean value of controls.

Fig. 3 Sodium concentration of nail clippings from CF patients and controls under one year of age. Symbols, see Fig. 2 —— values at different ages from the same healthy subject.

parametric tolerance limits it was found that more than 95% of the normal values were less than 3600 μg/g with a confidence of 90–95% and that more than 95% of the values for CF patients were more than 4000 μg/g with a confidence of 75–80% for subjects more than one year old.

Screening test in the neonatal period and in the first weeks of life

The results of 1322 screening tests are shown in Fig. 4. For comparison the values for older CF patients and for the two newborns with meconium ileus and proven CF are also presented in this figure.

but nails were also clipped from a few older children and adults (see Fig 2)

The screening series included nails from all 1322 babies born during the period 1 1—30 4 1969 At the time of nail clippings all babies were more than three days old 1124 of them were three to five days and the remaining 198 were six to ten days old

Nails from two newborn children with meconium ileus and proven CF were also analyzed These two infants did not belong to the screening population

All nail clippings, except in newborn infants were collected according to the routine described earlier (9) The children had no long periods of swimming in the week and no bath in the 24 hours preceding the clipping after mechanical removal of visible dirt the entire nail was soaked with distilled water by means of a gauze pad three hours later the nails were clipped and put into cleaned irradiation ampoules and mailed to the reactor center

The nails of the newborns were soaked with 70% alcohol instead of distilled water in an attempt to wash out more extrinsic sodium originating from the amniotic fluid (17) Otherwise the collection routine was exactly the same for the newborns as for the older subjects The care of the babies was essentially the same in the different nurseries

The irradiation and the detection of ^{24}Na in the nail clippings were done as reported in an earlier paper (9). Standards with known amounts of sodium were always irradiated together with the specimens The accuracy and precision of the activation analyses were checked via determinations of the sodium concentration in the international biological reference sample of kale powder which was occasionally irradiated together with the nail clippings The mean sodium value of six determinations was 2700 $\mu g/g$ with a standard deviation of five per cent (9) These values can be compared with the values obtained at other laboratories in the international study (1)

Statistical methods

The differences between the means of sodium concentration of the nails were examined with confidence intervals Such an interval contains the true difference i e the difference between the population means with a confidence of $100(1-\alpha)\%$

To investigate the diagnostic reliability the variations were examined with different kinds of tolerance intervals, intended to contain a certain proportion of the population One-sided intervals were used to give an upper limit for the controls and a lower limit for the CF patients. The one sided tolerance interval which on an average contains a $100(1-\alpha)\%$ proportion of the population was obtained as

$$\bar{x} + t_{1-\alpha}^{(n-1)} \sqrt{s^2 \left(1+\frac{1}{n}\right)}$$

where $t_{1-\alpha}^{(n-1)}$ is the upper $100(1-\alpha)\%$ point of the t distribution with $n-1$ degrees of freedom

In this formula the variable is assumed to be at least approximately normally distributed and the intervals might be misleading if this assumption is far from being fulfilled

A non-parametric tolerance interval which does not require any assumptions about the distribution, was also used. It is based on the theory of order statistics and gives intervals which with a certain confidence, contain at least a certain proportion Thus, the non-parametric intervals are wider than those described above One-sided intervals were obtained from tables by Jilek & Likar (7)

The newborn babies were classified according to four factors Nursery birth weight age at nail clipping and interval between soaking and clipping of nails. To obtain any differences for these factors analysis of variance (ANOVA) was used Since the number of children for each factor combination was not the same and was sometimes even zero several separate ANOVAs, three-way and two-way were carried out to cover the whole material An approximate method called unweighted means analysis was used (11). The advantage of using multi-way ANOVA including all factors of interest, before analyzing the factors separately by one-way ANOVA, is that interactions are revealed and that, in comparing the levels of one factor the influence of the other is eliminated

RESULTS

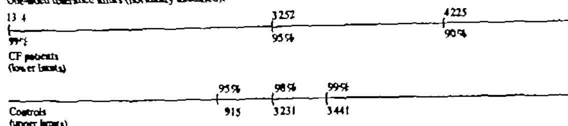
Analysis of nail clippings from patients with CF heterozygotes of the disease and healthy controls

Nails from 28 CF patients 22 heterozygotes and 52 controls were analyzed The mean values standard deviations ranges the 99% confidence intervals for the mean differences and the one sided tolerance limits are given in Table 1 The results are presented in a histogram (Fig 1) and plotted against age in Figs 2 and 3 For CF patients contra controls and heterozygotes the differences between the means were large and the ranges did not overlap The estimation of the one-sided tolerance limits revealed that in 95% of the nails of CF patients the sodium concentrations were above 3250 $\mu g/g$ and in 98% of the control nails they were below this value On the other hand the values for heterozygotes and controls were very similar and showed considerable overlapping

In all patients over one year of age the sodium concentration in the nail clippings was above 4000 $\mu g/g$ and in all controls it was lower than 3600 $\mu g/g$ From the non-

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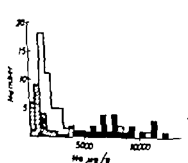




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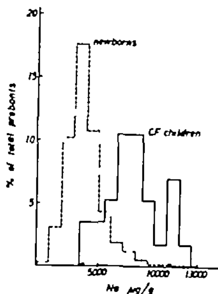


Fig 4 Histogram for sodium concentration of nail clippings from 1322 newborn infants and from 78 CF patients ---- newborns —— CF patients ● CF infants with meconium ileus

The eight children of the screening population with sodium concentration values in the nail clippings greater than 10000 µg/g were re-evaluated at the age of 6–18 months None had a family history of CF none had any clinical signs of CF and seven had normal sweat tests by the pilocarpine iontophoresis method The parents of the last one refused to let their child undergo a sweat test

The diagnostic accuracy was estimated by comparing the sodium concentrations of nails from the newborn healthy infants with those of older CF patients The result is shown in Table 2

The multi way analyses of variance revealed no significant differences in the values from nurseries B E or from the ward but the infants from nursery A showed slightly higher values than the others The explanation for this is not known Nursery A was excluded from the subsequent calculations

There seemed to be a sligt decrease in the sodium concentration of nail clippings from the fourth to the sixth day of life (Fig. 5)

The length of the interval between washing and clipping (2–12 hours) had no significant influence on the sodium concentration of the nails

Table 2 *Consequences of different borderlines of the concentration of sodium in nail clippings for the diagnosis of CF in newborn infants*

	% diagnosed correctly	
Na µg	CF patients n=78	Controls n=1322
3500	100	45
4500	93	75
6500	75	94
7000	65	96

The sodium concentration of nail clippings from pre-term babies (less than 37 weeks of gestation) and from small babies (below 2500 g) was found to be significantly lower than that of nail clippings from full term babies ($p<0\ 05$ and $<0\ 01$ respectively)

DISCUSSION

The possibility of diagnosing CF by INAA of nail clippings

The study shows that INAA of nail clippings has good diagnostic reliability

The one sided tolerance limits obtained with the assumption of normal distribution



Fig 5 Sodium concentrations of the nail clippings from newborns according to age at time of clipping —— own values ---- values of Seattle group —— values of Texas group

ndicate that 98% of normal persons have sodium concentrations in the nail clippings under 3250 μg/g and that 95% of CF patients have concentrations above this value after the first year of life

From the histograms (Figs. 1 and 4) it might be suspected however that the dis tribution for the controls is asymmetrical and has a positive skewness If this is the case the upper limits for the controls are too low On the other hand the distribution for the CF patients might be symmetrical but perhaps with a smaller kurtosis than the normal distribution This makes the lower limits obtained for the CF patients too low

Good reliability was also found from the non-parametric tolerance limits. The diagnostic ability of INAA of nail clippings is, in fact of the same order as that of the sweat test (12).

Thus we consider that INAA of nail clippings should be accepted as a diagnostic test for CF It will give every child with suspected symptoms of CF a chance of having the diagnosis tested even those in whom a sweat test is difficult to perform e g. for reasons of distance to a suitable hospital with facilities for this test.

A cost benefit analysis might be attempted from our results and with the following assumptions 1) All children with any symptoms of CF (12) which might amount to one out of twenty should be tested for CF by INAA 2) Practically all children with CF will show one of these symptoms at some time 3) CF has an incidence of 1 2000 new borns (2). 4) The cost of INAA of one nail is 50 Sw kr

With 100000 children born per year in Sweden this would mean about 5000 INAA per year From the non-parametric tolerance intervals we might calculate that less than five percent of normal persons have more than 3600 μg sodium/g nail, which thus would be exceeded in about 250 normal children and 50 CF patients These would have to undergo further confirmation of diagnosis. With this procedure about 50 CF patients per year would be given the diagnosis and about 2–3 would be missed. The proportions today are about 20 CF patients diagnosed and 30 missed per year The extra cost for this would be about 250000 Sw kr

The possibility of detecting heterozygotes by INAA of nail clippings

The sodium concentration in sweat increases with age (3) In this small series there was a tendency for subjects of older ages to have lower concentrations of sodium in their nails. This is in concordance with earlier findings (10) One possible explanation is that the capacity of the nail to absorb sodium de teriorates with age. This might also explain the finding that the heterozygotes of this study all of whom were adults had lower sodium concentrations in their nails than the controls who were mainly children This is also in agreement with earlier findings (4 14 17) Anyway heterozygotes cannot be detected by determining the sodium concen tration in the nails

The possibility of using INAA as a screening test in the neonatal period

In newborns there are three factors that influence the sodium concentration of the nails 1) the nails have been thoroughly contami nated with the sodium rich amniotic fluid for a long period 2) the sweat glands are func tioning at a low rate and 3) the sweat of all newborns has a higher sodium concentration in the first three days of life (16)

Nails from normal newborns have their maximum concentrations of sodium and chlorine at the time of birth These concentrations decrease to about half the value within the first three days of life (4 17) and there seems to be a further fall in the concentrations during days four–six of life

Our two values and about 25 results from the literature (14 17) indicate that the concentrations of sodium in the nails of children

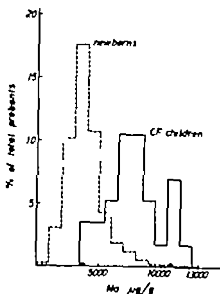


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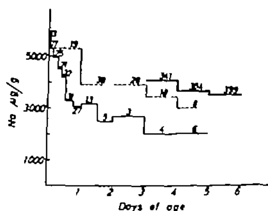


Fig 5 Sodium concentrations of the nail clippings from newborns according to age at time of clipping —— own values - - - - values of Seattle group —— values of Texas group

ndicate that 98% of normal persons have sodium concentrations in the nail clippings under 3250 μg/g and that 95% of CF patients have concentrations above this value after the first year of life

From the histograms (Figs 1 and 4) it might be suspected however that the distribution for the controls is asymmetrical and has a positive skewness If this is the case the upper limits for the controls are too low On the other hand the distribution for the CF patients might be symmetrical but perhaps with a smaller kurtosis than the normal distribution. This makes the lower limits obtained for the CF patients too low

Good reliability was also found from the non-parametric tolerance limits. The diagnostic ability of INAA of nail clippings is in fact, of the same order as that of the sweat test (12)

Thus, we consider that INAA of nail clippings should be accepted as a diagnostic test for CF It will give every child with suspected symptoms of CF a chance of having the diagnosis tested even those in whom a sweat test is difficult to perform e g for reasons of distance to a suitable hospital with facilities for this test.

A cost benefit analysis might be attempted from our results and with the following assumptions. 1) All children with any symptoms of CF (1?) which might amount to one out of twenty should be tested for CF by INAA 2) Practically all children with CF will show one of these symptoms at some time 3) CF has an incidence of 1 2000 new borns (2). 4) The cost of INAA of one nail is 50 Sw kr

With 100000 children born per year in Sweden this would mean about 5000 INAA per year From the non-parametric tolerance intervals we might calculate that less than five percent of normal persons have more than 3600 μg sodium/g nail which thus would be exceeded in about 250 normal children and 50 CF patients. These would have to undergo further confirmation of diagnosis. With this procedure about 50 CF patients per year would be given the diagnosis and about 2–3 would be missed. The proportions today are about 20 CF patients diagnosed and 30 missed per year The extra cost for this would be about 250000 Sw kr

The possibility of detecting heterozygotes by INAA of nail clippings

The sodium concentration in sweat increases with age (3) In this small series there was a tendency for subjects of older ages to have lower concentrations of sodium in their nails This is in concordance with earlier findings (10) One possible explanation is that the capacity of the nail to absorb sodium deteriorates with age This might also explain the finding that the heterozygotes of this study all of whom were adults had lower sodium concentrations in their nails than the controls who were mainly children This is also in agreement with earlier findings (4 14 17). Anyway heterozygotes cannot be detected by determining the sodium concentration in the nails

The possibility of using INAA as a screening test in the neonatal period

In newborns there are three factors that influence the sodium concentration of the nails 1) the nails have been thoroughly contaminated with the sodium rich amniotic fluid for a long period 2) the sweat glands are functioning at a low rate and 3) the sweat of all newborns has a higher sodium concentration in the first three days of life (16)

Nails from normal newborns have their maximum concentrations of sodium and chlorine at the time of birth These concentrations decrease to about half the value within the first three days of life (4 17) and there seems to be a further fall in the concentrations during days four–six of life

Our two values and about 25 results from the literature (14 17) indicate that the concentrations of sodium in the nails of children

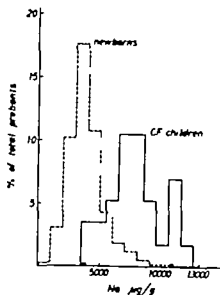


Fig 4 Histogram for sodium concentration of nail clippings from 1322 newborn infants and from 28 CF patients ---- newborns —— CF patients ● CF infants with meconium ileus

The eight children of the screening population with sodium concentration values in the nail clippings greater than 10000 μg/g were re-evaluated at the age of 6–18 months None had a family history of CF none had any clinical signs of CF and seven had normal sweat tests by the pilocarpine-iontophoresis method The parents of the last one refused to let their child undergo a sweat test

The diagnostic accuracy was estimated by comparing the sodium concentrations of nails from the newborn healthy infants with those of older CF patients The result is shown in Table 2

The multi way analyses of variance revealed no significant differences in the values from nurseries B E or from the ward but the infants from nursery A showed slightly higher values than the others The explanation for this is not known Nursery A was excluded from the subsequent calculations

There seemed to be a sligt decrease in the sodium concentration of nail clippings from the fourth to the sixth day of life (Fig. 5)

The length of the interval between washing and clipping (2–12 hours) had no significant influence on the sodium concentration of the nails

Table 2 *Consequences of different borderlines of the concentration of sodium in nail clippings for the diagnosis of CF in newborn infants*

	% diagnosed correctly	
Na μg	CF patients $n=28$	Controls $n=1322$
3500	100	45
4500	93	75
6500	75	94
7000	65	96

The sodium concentration of nail clippings from pre term babies (less than 37 weeks of gestation) and from small babies (below 2500 g) was found to be significantly lower than that of nail clippings from full term babies ($p<0$ 05 and <0 01 respectively)

DISCUSSION

The possibility of diagnosing CF by INAA of nail clippings

The study shows that INAA of nail clippings has good diagnostic reliability

The one sided tolerance limits obtained with the assumption of normal distribution

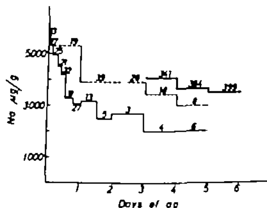


Fig 5 Sodium concentrations of the nail clippings from newborns according to age at time of clipping —— own values ---- values of Seattle group —— values of Texas group

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with CF are about the same in the first two weeks of life as later in childhood Because of this we made a calculation of the diag nostic ability by comparing the sodium con centrations of the nails of newborn babies with those of older CF children

As could be expected the diagnostic ability seemed to be better in our study on new borns who were four days of age or more (Table 2) than in a study on three-day old infants (14) The test cannot however be used as for screening during the first week of life If no newborns with CF are to be missed a borderline at about 3500 μg/g must be chosen and more than half of the newborns would have such high values that they would have to undergo further testing If seven percent were allowed to remain un diagnosed (borderline 4500 μg/g) there would still be one out of four newborns who would have high values A manageable num ber of children to be tested further (i e a few percent) would give a borderline at about 7000 μg/g and lead to a missed diagnosis in one out of three

Thus INAA of nail clippings is only of limited value during the first week of life The contaminative sodium from the amniotic fluid disappears rapidly however and analy sis of nail clippings might be of diagnostic value after only a few weeks But there are many physiological alterations during the first year of life that may interfere with the sodium concentration in the nails e g a low sweating rate and low sodium concentration of sweat The few normal and pathological values below one year of age in our study are not sufficient to allow any conclusions to be drawn about the youngest age at which INAA of sodium in nail clippings might be used as a diagnostic tool

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Table 1 *Bacterial flora of mouth stomach and duodenum of infants with gastroenteritis and of infants with healthy alimentary tract*

"Oral-type" =species regarded as normal flora of the mouth "Faecal-type"=species regarded as normal flora of faeces

Group	No. of patients	Mouth		Stomach			
		"Faecal-type"	*Candida albicans*	No growth	"Oral-type	Faecal-type	*Candida albicans*
Gastroenteritis	39	12	7	13	13	7	13
Control	20	13	2	10	7	7	2

to the gastrointestinal tract at the time of investigation. They included patients with meningomyelocoele (12), hydrocephalus (1), head injury (1), tracheo-oesophageal fistula (1), oesophageal diverticulum (1), orchidopexy (1), and three infants several weeks after bowel surgery for meconium ileus (1), meconium peritonitis (1) and necrotizing enterocolitis (1). Seven of these 20 patients had received parenteral antibiotics in the 7 days prior to investigation. They included methicillin and gentamicin (2), chloramphenicol and gentamicin (1), erythromycin and cephaloridine (1) cephaloridine alone (2) and ampicillin (1). Antifungal agents had not been used in any

Ages of the gastroenteritis patients were evenly distributed throughout the 12 months. A slightly higher proportion of control infants was less than 2 months old.

Methods

All investigations were performed after rehydration and within 48 hours of admission. Glucose solution (5%) was the only oral feeding given in hospital prior to the specimens being obtained.

Sampling

Faecal and throat swabs were taken. Saliva was aspirated with a sterile syringe. A sterile radio-opaque rubber catheter was then passed into the stomach. The first 2 ml of gastric aspirate were discarded and the remainder used for culture. The catheter was manipulated into the 4th part of the duodenum and position checked by fluoroscopy Then first 2 ml of duodenal aspirate were discarded and the remainder used for culture

Bacteriology

Specimens were plated immediately or after refrigeration for one hour Two drops (0 05 ml) of undiluted saliva, stomach or duodenal contents were placed on each of the following media, horse blood agar McConkey agar Rogosa agar deoxycholate agar Sabouraud agar incubated aerobically at 37°C and on horse blood agar and Rogosa agar incubated anaerobically with the addition of 5–10% carbon dioxide Faecal swabs were plated on the same media. All specimens were incubated in selenite broth for 48 hours. Plates were examined after incubation for 24 hours, and again at intervals for at least seven days. Two drops of each specimen were dried at room temperature on a glass slide. A Gram stain of the smear acted as a check on the adequacy of culture. A standard plating technique that allowed semi-quantitative estimation of numbers present was used (6). Microbial counts using the technique of Miles et al. (14) were compared with the results of plating, in order to assess numbers of organisms present. Yeast counts were checked by direct count under high power in a hemacytometer All bacteria isolated were subcultured and identified according to criteria described by Gibbs & Skinner (9). *E. coli* strains were typed serologically using commercial polyvalent and monovalent antisera (BDH).

Table 2. *Occurrence of micro-organisms unusual in the upper alimentary tract*

	39 infants with gastroenteritis				20 controls			
	Any level	Mouth	Stomach	Duodenum	Any level	Mouth	Stomach	Duodenum
E. coli	13	9	5	11	10	6	5	4
Klebsiella sp.	2				1	1		
Citrobacter sp	1	1	1	1	2	2		
Pseudomonas sp	1		1	1	3	3	3	2
Proteus sp	1		1	1	3	2	2	
Strep. faecalis	1	1		1	1	1	1	1
Enterobacter sp.					2	1	1	
Candida albicans	14	9	13	12	3		2	2
Candida parapsilosis			2	2				

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MICROBIAL FLORA OF STOMACH AND SMALL INTESTINE IN INFANTILE GASTROENTERITIS

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ABSTRACT Bishop R F Barnes, G L and Townley R. R. W (Department of Gastroenterology Royal Children's Hospital, Melbourne and Department of Paediatrics, University of Melbourne Victoria, Australia) Microbial flora of stomach and small intestine in infantile gastroenteritis. Acta Paediatr Scand, 63: 418 1974.—Microbial flora of mouth stomach duodenum and faeces was examined in 39 infants with acute gastroenteritis. Only one recognised pathogen, *Salmonella typhimurium* was isolated. No enteropathogenic strains of *Escherichia coli* were isolated. There was an increase in abundant growth of *Candida albicans* in stomach and small intestine of infants with gastroenteritis compared with the control group.

KEY WORDS: Gastroenteritis, *C albicans*, *E coli*, stomach small intestine infants

Gastroenteritis in infancy may be caused by a variety of microbial pathogens However in most patients no recognised pathogen is isolated from faeces or throat swab A recent review concludes that further studies of throat and faecal flora will not contribute to knowledge of the aetiology of gastroenteritis in infants (5)

One possible explanation for the previous failures to isolate a pathogen is that the pathogen has rarely been sought at the appropriate levels of the intestine i e the stomach and small intestine Thomson in 1955 (18) was first to demonstrate infection by *Escherichia coli* in the upper small intestine in babies with gastroenteritis Recent studies show colonisation of upper small intestine by microbial pathogens in human cholera (10) in acute adult diarrhoea in the tropics (11) and in diarrhoeal disease in pigs due to *E coli* (16)

The aim of this study was to examine in detail the microbial flora of throat gastric duodenal and rectal contents during the acute stage of gastroenteritis in infants aged less than one year

MATERIALS AND METHODS

Patients and controls

Gastroenteritis was defined as a febrile illness of less than 10 days duration associated with diarrhoea and vomiting and with no other evident cause for symptoms.

Thirty nine infants less than a year of age admitted to the Royal Children's Hospital Melbourne, with gastroenteritis were studied Three infants whose inclusion would have made the series a consecutive one, were excluded because parental permission for the investigations was not obtained There were 25 males and 14 females. None of the children was malnourished or underweight when rehydrated Fifteen infants required intravenous therapy Nine infants had had symptoms for less than 48 hours, 29 for 2–7 days and one for 9 days.

Sixteen of the 39 had received varying courses of antibiotics during the seven days prior to sampling. These included penicillin (6) neomycin (5), erythromycin (1), trimethoprim and sulphamethoxazole (1) penicillin and colistin (1) All were given by mouth except colistin methicillin and kanamycin. None had had antifungal agents.

The 70 control infants were patients under 1 year of age from surgical wards. None had symptoms referable

Table 4 *Occurrence of C albicans in relation to prior antibiotics*

	39 infants with gastroenteritis			20 controls	
Level of intestine	No antibiotics (23 patients)	Penicillin only (6 patients)	Broad spectrum antibiotics (10 patients)	No antibiotics (13 patients)	Broad spectrum antibiotics (7 patients)
Any of the 4 sites	10=44%	1=17%	3=30%	2=15%	1=14%
Mouth	6	1	2	1	1
Stomach	10	1	2	1	1
Duodenum	9	1	2	2	0
Rectum	4	0	2	0	1

The occurrence of *C albicans* was not increased by administration of antibiotics prior to sampling (Table 4). Of the 39 gastroenteritis patients the group receiving oral broad spectrum antibiotics showed a lower incidence of *C albicans* (3/10) than the group not given antibiotics (10/32) Table 4 shows that *C albicans* was present more often in stomach and duodenum that in saliva or rectal swabs It was present in stomach in pure culture in 9 patients. Mycelium was sometimes seen in Gram-stained smears of stomach and duodenal contents but no penetration of duodenal mucosa was detected histologically in biopsy specimens.

The numbers of *C albicans* cultured from different levels of the alimentary tract are shown in Table 5 The count indicating abundant growth is assessed by ourselves and by others (4) at $\geq 10^3$ yeast cells/ml. Of the 14 patients with gastroenteritis from whom *C albicans* was isolated 10 showed abundant growth in stomach or duodenum By contrast, only 1 infant in the control gruop yielded abundant growth at these levels. The difference between abundant growth in gastroenteritis and in the control group is statistically significant ($0.05>p>0.025$).

Virology

Duodenal contents of 10 patients with gastroenteritis were examined for viruses by conventional cell culture techniques Sabin polio vaccine and an Echo virus were isolated from two separate patients.

DISCUSSION

The many reviews of sporadic gastroenteritis in children conclude that in the majority of patients no viral or bacterial pathogen can be isolated from mouth or faecal swabs (15) By definition gastroenteritis involves inflammation of the stomach and small bowel but no previous studies have adequately examined microbial flora at these levels during the acute stage of the disease Instead the published results are from specimens obtained some time after the onset of the illness (3) at postmortem (1 7) or describe the presence of *E. coli* only (18)

This study presents new information relating to the extent of microbial flora in infantile gastroenteritis As with previous studies no recognised intestinal pathogens were isolated from the majority of patients. In particular there was little to implicate *E. coli* as an important cause of infantile diarrhoea in this city

Table 5 *Candida albicans in the alimentary tract quantitative results*

	14 gastroenteritis patients		3 control patients	
Level of alimentary tract	Counts $\geq 10^3$/ml	Counts $<10^3$/ml	Counts $\geq 10^3$/ml	Counts $<10^3$/ml
Mouth	2	7	1	1
Stomach	10	3	1	1
Duodenum	8	4		2
Rectum		4	1	

Duodenum			
No growth	Oral-type	Faecal-type	*Candida albicans*
6	24	14	12
9	5	6	2

The ability of strains to form enterotoxin was tested in ligated loops of rabbit intestine (7) Yeasts were identified by biochemical tests and colonial morphology described by Martin et al (13) *C albicans* was additionally identified by ability to form germ tubes (17)

RESULTS

Results from faecal specimens are not tabulated in detail *Salmonella typhimurium* was isolated from the faeces of one infant with gastroenteritis All other faecal specimen from both groups of infants yielded only normal faecal flora

Table 1 lists the microbial flora of mouth stomach and duodenum of children with gastroenteritis and of the control group Specimens yielding no growth were more common from control infants than from gastroenteritis patients The organisms most commonly isolated from both groups were of oral-type i e they were species regarded as normal flora of mouth nose and throat and comprised streptococci of the viridans group *Neisseria* sp *Staphylococcus albus* *Veillonella* sp *Corynebacterium* sp *Lactobacillus* sp (aerobic and anaerobic) and *Haemophilus* sp In any one child the same species of oral type were usually present in mouth stomach and duodenum

Organisms regarded as faecal type were isolated from one of the three levels of the upper alimentary tract in 15/39 patients with gastroenteritis and in 14/20 control patients The occurrence of each species is listed in Table 2 With the exception of *E coli* all faecal type organisms isolated were present in numbers $<10^4$ organisms/ml This is not considered by ourselves or others (8) to be a significant level of growth in the upper intestine

Table 2 shows that *E. coli* was isolated from one of the three levels of the upper alimentary tract in 13/39 gastroenteritis patients (33%) and in 10/20 control infants (50%) The incidence was not affected by prior use of broad spectrum antibiotics in either group (Table 3) No strain reacted with antisera to the recognised enteropathogenic serotypes *E coli* occurred more frequently in significant number ($\geq 10^4$/ml) in stomach and small intestine of gastroenteritis patients (4/13) than of control infants (1/10) None of these strains produced an enterotoxin when tested in ligated loops of rabbit ileum

The most marked difference between gastroenteritis patients and control patients was the frequency with which *C albicans* was isolated from patients with gastroenteritis (Table 2) It was isolated from at least one level of the intestinal tract in 14/39 patients with gastroenteritis and from only 3/20 patients in the control series No infant in either group had clinical thrush This difference in incidence is not statistically significant ($0.1>p>0.05$) In the gastroenteritis group one patient grew *C tropicalis* from faeces only and two patients grew *C parapsilosis* from stomach and duodenum

Table 3 *Occurrence of E coli in mouth stomach or duodenum in relation to prior antibiotics*

	39 infants with gastroenteritis		20 controls	
	No of patients	*E coli*	No of patients	*E coli*
No antibiotics	23	9=39%	13	7=54%
Broad spectrum antibiotics	10	3=30%	7	3=43%
Penicillin only	6	1=17%	0	0

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MICROBIAL FLORA AND DISACCHARIDASE DEPRESSION IN INFANTILE GASTROENTERITIS

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ABSTRACT Barnes, G. L., Bishop, R. F. and Townley, R. R. W. (Department of Gastroenterology, Royal Children's Hospital, Melbourne and Department of Paediatrics, University of Melbourne, Victoria, Australia). Microbial Flora and Disaccharidase Depression in Infantile Gastroenteritis. Acta Paediatr Scand, 63: 423, 1974.—In infants with acute gastroenteritis, disaccharidase activity in duodenum is depressed in a majority of patients. There is a statistically significant association between abundant growth of *Candida albicans* in the duodenum and depression of lactase activity at the same level.

KEY WORDS: Gastroenteritis, *E. Coli*, *C. albicans*, disaccharidase, infants

A survey of microbial flora of infants with acute gastroenteritis revealed *Candida albicans* growing abundantly in stomach and duodenum in one-third of the patients (3). This incidence was higher than in a control group examined at the same time, and higher than in previous surveys of mouth and rectal flora in infantile gastroenteritis.

Depressed disaccharidase levels were observed in a majority of patients, usually in infants showing histological changes in duodenal mucosa (1).

When results of enzyme assays were compared with microbial flora of duodenal contents, an association was observed between disaccharidase depression and growth of *C. albicans*. This paper discusses the nature of this association.

MATERIAL AND METHODS

Thirty-one infants aged less than one year were examined by duodenal biopsy and intubation. There were 22 males and 9 females. All weighed 4.0 kg or more and had acute gastroenteritis at the time of sampling. None of the children was malnourished or underweight when rehydrated.

Detailed descriptions of criteria for selections of patients, techniques of duodenal biopsy and intubation, and methods used for enzyme assay and microbial culture are recorded elsewhere (1, 3).

When assessing results of microbial culture, the levels considered by ourselves and by others as indicating growth at the site sampled were $\geq 10^{5}$ yeasts/ml (6); $\geq 10^{4}$ *Escherichia coli*/ml (7).

The levels of disaccharidase activity regarded as normal were $\geq$9.0 units maltase/g wet weight tissue, $\geq$3.5 units/sucrase/g wet weight, $\geq$1.1 units lactase/g wet weight. Infants were regarded as having disaccharidase depression when levels of at least two enzymes were below these normal values.

These levels were chosen because they all fell at the 5th percentile of values obtained in a consecutive series of 100 children initially investigated for chronic diarrhoea or failure to thrive. Duodenal mucosal histology was normal in all, and no organic basis for their symptoms was discovered. The chosen levels are probably lower than those that would be found were a group of healthy children to be studied. Such a study is precluded for ethical reasons.

Statistical analysis of the results, comparing microbial flora with enzyme levels, made use of the chi-square test and Yates' correction for small numbers.

RESULTS

Detailed descriptions of the results of culture and of disaccharidase assays are presented elsewhere (3, 1).

A finding of interest was the increased incidence of *C albicans* in infants with gastroenteritis compared with the control group This difference in incidence was not statistically significant However if degree of growth is regarded as important as it is in interpretation of quantitative results from urine specimens then there was a statistically significant increase in abundant growth of yeast in infants with gastroenteritis compared with the control group The increase was not related to prior antibiotic therapy nor to oral thrush The yeast was isolated early in the disease often only 48 hours after onset of symptoms It was present in large numbers in stomach and duodenum sometimes in pure culture forming tangled mycelium in Gram-stained smears Its presence in stomach and duodenum was not due to contamination by swallowed saliva since *C albicans* was found more often and in larger numbers in stomach and duodenum than in the mouth

The importance of *C albicans* in relation to aetiology of the disease is not clear It is possible that it was primarily involved in some infants This has previously been suggested by Kozinn & Taschdjian (12) It is also possible that the yeast is opportunistically colonising gut already damaged by another agent (presumably viral) The yeast might then contribute to prolongation of clinical symptoms A controlled trial of the use of antifungal agents in treatment of acute gastroenteritis in infants would elucidate the role of *C albicans* in this disease

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DISCUSSION

Despite much recent interest in duodenal disaccharidase activity in disease no previous studies have related enzyme levels to microbial flora of the duodenum in man. Coello-Ramirez et al (5) described a linear increase in duodenal bacterial counts with increasing severity of carbohydrate intolerance. The changes they observed in bacterial flora were possibly secondary to gut damage since the majority of infants studied had diarrhoea of more than 10 days duration.

This study has examined disaccharidase levels and microbial flora of the duodenum during an earlier acute phase of gastroenteritis. The observed disaccharidase depression could not be explained by growth of bacterial pathogens including enterotoxin producing strains of *E. coli*, nor was it explained by a non-specific overgrowth of normal bacterial flora. However there was an abundant growth of *C albicans* in stomach and duodenum of many of the infants with disaccharidase depression. In particular there was a statistically significant association between growth of *C albicans* and lactase depression.

There are two possible explanations for this association. Firstly an unknown agent could cause initial disaccharidase depression. The resulting unabsorbed sugar in the gut lumen might then enhance growth of *C albicans* as a secondary phenomenon. Blacklow and his colleagues (?) in a recent discussion of acute infectious non-bacterial gastroenteritis describe transient malabsorption of lactose. However malabsorption of lactose alone would not enhance growth of *C albicans* since the yeast does not ferment this sugar. Unabsorbed sucrose or glucose in the gut lumen could stimulate growth of the yeast (9) but the non-bacterial infectious agent described by Blacklow et al (2) did not affect absorption of glucose and there was no correlation in this study between presence of these sugars in the diet and later isolation of the yeast.

A second explanation for association between *C albicans* and disaccharidase depression is that growth of the yeast in the gut lumen reduces enzyme activity at the same level. Experimental support for this hypothesis was obtained by injecting strains of *C albicans* isolated from infants with gastroenteritis into isolated loops of small bowel of infant rabbits. There was a statistically *significant* decrease of lactase activity in test loops compared with matched control loops inoculated with sterile broth ($0.025 > p > 0.01$). These results will be reported elsewhere in detail (10).

Another instance of association between *C albicans* and disaccharidase depression has been described in children with defective cellular immune function. Six such children were found to have reduced lactase sucrase and maltase levels in duodenum (8). Although cultures of duodenal juice are stated to be negative for intestinal pathogens all six children had suffered recurrent monilial stomatitis. It is possible that the primary defect in cellular immune function in these children allowed colonisation of the upper alimentary tract by *C albicans* leading to depression of disaccharidase activity at the same level.

The evidence presented above is still equivocal. It is possible that depression of disaccharidase activity in duodenum of infants with acute gastroenteritis is due to an unknown agent (presumably viral). It is also possible that growth of *C albicans* in an already damaged small intestine could initiate or perpetuate depression of intestinal disaccharidase activity. If this is so then other disease states favouring colonization of upper small intestine by *C albicans* might also be expected to show depression of disaccharidase activity.

ACKNOWLEDGEMENTS

We wish to thank Mr Max Murray for estimations of disaccharidase activity

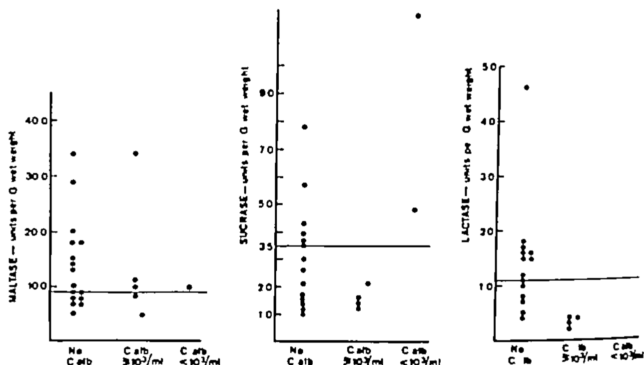


Fig 1 Maltase sucrase and lactase levels correlated with numbers of *C albicans* grown from duodenal contents of infants with acute gastroenteritis. C alb = *Candida albicans*

Table 1 shows the disaccharidase levels in duodenum in relation to growth of *C albicans* and *E coli* at the same level Of the 31 patients examined 18 had disaccharidase depression Eight of these patients grew *C albicans* in abundance in the duodenum Thirteen patients had normal disaccharidase activity but only one grew *C albicans* in large numbers This same patient later developed persisting sugar intolerance but disaccharidase levels were not reestimated The association between disaccharidase depression and growth of *C albicans* was not statistically significant ($0.1 > p > 0.05$) However when individual disaccharidase levels were analysed (Fig. 1) there was a statistically significant association between lactase depression and growth of *C albicans* $0.01 > p > 0.001$) The results for maltase and sucrase depression were not statistically significant ($0.2 > p > 0.1$).

The results comparing disaccharidase depression and growth of *E coli* in duodenum are listed in Table 1 None of the *E coli* strains produced enterotoxin when tested in ligated rabbit gut (4) The incidence of *E coli* in infants with abnormal disaccharidase levels is the same as in infants with normal enzyme activity There is thus no association between growth of *E coli* and alteration in disaccharidase levels

Table 1 *Disaccharidase activity in duodenum in relation to growth of Candida albicans and Escherichia coli*

Disaccharidase activity in duodenum	No of patients	*C albicans*		*E coli*	
		$\geq 10^3$/ml	$<10^3$/ml	$\geq 10^4$/ml	$<10^4$/ml
Normal	13	1	1	1	4
Abnormal	18	8	1	2	3

Total number of patients = 31

DIAGNOSTIC USEFULNESS OF THE NITROBLUE TETRAZOLIUM DYE REDUCTION TEST IN A CHILDREN'S HOSPITAL

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ABSTRACT Marks, M. I. and Dery P (Dept. of Pediatrics, Infectious Disease, McGill University-Montreal Children's Hospital Research Institute, Montreal, Canada). Diagnostic usefulness of the nitroblue tetrazolium dye reduction test in a children's hospital. Acta Paediatr Scand, 63: 427 1974.—The usefulness of the classical and stimulated nitroblue tetrazolium dye (NBT) reduction test in the diagnosis of pediatric infectious disease was evaluated. One hundred and fifty-two patients were studied. An overall correlation of increased phagocytosis with bacterial infections was noted, but the test was not judged useful in differentiating most infections that were difficult to diagnose by other clinical and laboratory criteria. There were 4.8% false negatives among 21 proven bacterial infections studied before treatment, and 27% false positives in 26 proven viral infections. Caution is urged in the use of the NBT test for diagnosis and treatment of the individual patient. When carefully performed and controlled, the NBT test should serve to help confirm clinical diagnoses and to screen for certain leukocyte bactericidal deficiencies.

KEY WORDS: Nitroblue tetrazolium dye reduction test, phagocytosis

Park et al. (17) described increased nitroblue tetrazolium dye (NBT) reduction by neutrophils of patients with acute bacterial infections. These results have been confirmed by other investigators (7 8 9 12, 13) however practical and theoretical limitations of the test have been described. (1 2 3 4 5 11 14 15 19 20 21 23) We report our experience over the past year which emphasizes the restricted role of this test in the differential diagnosis of common pediatric infectious diseases in a hospital setting.

MATERIALS AND METHODS

House officers and attending staff at the Montreal Children's Hospital were informed about the potential usefulness and known limitations of the NBT test and were invited to request the procedure in clinical situations where they thought the results might be useful. Specimens were processed within ½–1 hour of request. One and one half ml of blood was drawn by the technician or house officer into a 2½ cc disposable plastic syringe containing 0.2 ml heparinized saline (200 μ/ml). The NBT test was performed as previously described (12). Eight drops of heparinized blood were mixed in a small disposable plastic tube with four drops of Krebs-phosphate buffer pH 7.4 which contained 200 mg of glucose per 100 ml, and four drops of a 0.1% solution of NBT in isotonic saline. The mixture was gently mixed by hand and was incubated for 15 minutes in a water bath at 37°C. A gentle shake was given every 5 minutes. Glass coverslip preparations were then Wright stained and the percentage of neutrophils containing a definite mass of formazan (reduced NBT) was determined.

Towards the latter half of the investigation safranin counterstain (smear fixed in 99% methanol ×1 minute, air dried and stained x 2 minutes with Gram's safranin) was used instead of Wright's stain. In addition to the usual NBT test (12) endotoxin stimulation was also performed (18) 0.05 ml *E.* coli lipopolysaccharide (Westphal) endotoxin (courtesy of Dr H. Robson) was added to 0.5 ml. heparinized blood and incubated at 37°C×

This study was supported by a grant from the McGill University Montreal Children's Hospital Research Institute

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Table 1 *158 NBT tests (152 patients)*

Type of Infection	Proven acute bacterial		Probable acute bacterial		Proven nonbacterial	Probable nonbacterial
Time tested	Pre Rx	Post Rx	Pre Rx	Post Rx		
No pats. tested	21	30	9	14	[illegible]6	58
Range of NBT score	1–85%	1–84%	11–56%	–38%	1–76%	1–78%
Mean NBT	41.5	19 6	[illegible]6.5	11 0	14 8	7 6
False pos. (>15%)					7	5
False neg (≤15%)	1	16	[illegible]	9		

10 minutes An aliquot (0 15 ml) was then incubated with 0 15 ml phosphate buffered saline pH 7 4 and incubated at 37°C×5 minutes and stained as above Clinical and laboratory data were used to classify the patients diagnoses as previously outlined (12).

RESULTS

One hundred and sixty five patients were studied Thirteen tests were not reported due to improper sampling extraordinary delay in processing the specimens extreme neutropenia and technical errors Results in 152 patients are listed in Table 1 Only 21 proven bacterial infections were tested before therapy Most of the tests were requested on patients already receiving antibiotics or in illnesses where etiological diagnoses were difficult to establish by clinical or laboratory means Although the majority of these patients were cultured and/or tested serologically for common pathogenic bacteria and viruses the exact cause of infection was often not determined

The numbers of false positives and false negatives are presented in Table 1 using 15% as the upper limit of NBT positive neutrophils in normal noninfected control subjects (32 determinations) Ten percent of the tests were falsely decreased in proven and probable bacterial infections studied before initiation of antibiotherapy The false negatives were encountered with proven streptococcal pharyngitis abcesses and salmonellosis and with probable bacterial pneumonia and otitis media.

The NBT was falsely negative in 56 8% of tests performed during antibiotic therapy These patients had the following diagnoses. osteomyelitis abcesses probable bacterial pneumonia and salmonellosis They received cloxacillin penicillin ampicillin and erythromycin therapy The patients with elevated NBT s had diagnoses including brucellosis, *Hemophilus influenzae* bacteremia streptococcal pharyngitis abcesses furunculosis meningococcal meningitis *H influenzae* meningitis *S pneumoniae* bacteremia and meningitis osteomyelitis scarlet fever arthritis empyema pneumonia and otitis media. Their antibiotic therapy included the penicillins listed above and tetracycline and streptomycin The false negatives were not associated with specific diagnoses or antibiotics

In proven and probable nonbacterial infections 14 3% of NBT's were falsely positive These patients were not on antibiotics and their diagnoses included aseptic meningitis rheumatoid arthritis toxic synovitis viral pneumonia exfoliative dermatitis and nonbacterial gastroenteritis The patients with expectedly low NBT s had pharyngitis, aseptic meningitis erythema multiforme, asthma, bronchopneumonia bone cyst infectious mononucleosis uremic pericarditis measles and giant cell pneumonia

Stimulation with endotoxin increased the percentage of positive phagocytes but the stimulated count was not significantly different in bacterial and nonbacterial infections and could not be used to differentiate these

Serial NBT's were performed during antibiotherapy in three patients Unlike in most

acute bacterial infections, the NBT's in two patients with brucellosis receiving tetracycline and one with osteomyelitis treated with cloxacillin returned to normal (≤15%) over a period of twenty to thirty days These types of infections usually require prolonged antibiotic therapy

DISCUSSION

The data presented in this study does not support the recent enthusiasm for a new test to differentiate bacterial from nonbacterial infections" Rather the test should be used as a guide to the type of infection present. Considered along with historical and physical findings the white blood cell count and, in certain situations other tests the NBT should be confirmatory in most cases of bacterial and viral infections It would be unwise to use the test as the sole criteria for treating a patient whose diagnosis is obscure A negative result seems more useful than a positive one Only one patient of the 75 with an NBT<15 before therapy had a proven bacterial infection. False positives noted in patients with infectious mononucleosis and viral meningitis were explained by concurrent streptococcal pharyngitis and otitis media respectively Further study is necessary to evaluate the role of serial NBT's to guide chemotherapy of brucellosis, osteomyelitis and similar infections Our results in three patients are suggestive of this possibility

The list of factors associated with false positives and false negatives continues to grow and includes the patient's age (11) prednisone (15) malaria (3) other parasitic diseases (4) vinblastine (2) alcohol (23) surgery (19), influenza (5) burns (21) and oral contraceptives (16). To this we would add technical difficulties and errors in interpretation of slides. Safranin staining may help obviate some of these difficulties Elucidation of the stimulatory and inhibitory factors and of the intracellular mechanisms involved in NBT reduction seems essential for its effective clinical application. The authors have heard of many disappointed efforts at introducing the test into routine laboratories. These failures usually illustrate the need for careful training of laboratory personnel consistency of technique and an initial 3–4 month period of practice Only then can the NBT serve the clinician as a clue to diagnosis. With the addition of endotoxin stimulation the test can also screen for a number of bactericidal deficiencies of circulating leukocytes.

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The authors gratefully acknowledge the technical assistance of Miss Marie L. Juneau

ADDENDUM

Three reports were recently presented at the thirteenth Inter-Science Conference (6 10 22) on antimicrobials and chemotherapy These reports stress the limited usefulness of the NBT test in the differentiation of bacterial and viral infections. They are in agreement with our data and support the conclusions that caution against the wide spread introduction of this test as a specific diagnostic tool

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REFERENCE VALUES FOR CORD BLOOD LIPID AND LIPOPROTEIN CONCENTRATIONS

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ABSTRACT Dyerberg, J., Hjørne, N., Nymand, G. and Olsen, J. S. (Department of Clinical Chemistry A, Rigshospitalet, Copenhagen, Department of Clinical Chemistry and Department of Gynaecology and Obstetrics, Aalborg Sygehus, Aalborg, Denmark). Reference value for cord blood lipid and lipoprotein concentrations. Acta Paediatr Scand 63: 431 1974.—Cord blood lipid and lipoprotein concentrations in 120 newborns 60 boys and 60 girls, have been determined in order to evaluate reference values for these components. The lipoprotein analyses were performed with an electrophoretic technique, which enables correction for the difference in lipid composition of the lipoproteins in the quantification procedure. The lipid analyses revealed a small, but statistically significant higher phospholipid concentration in girls than in boys ($p<0.05$). A similar difference in cholesterol concentration was not statistically significant ($p>0.05$). No sex difference was found in lipoprotein concentrations. Our results were generally found in accordance with the few other previous studies on lipoproteins in cord blood. The results were also directly comparable to those in a recent study on the LDL-cholesterol concentration in cord blood (12). This supports the assumption that the lipoprotein composition in newborns is similar to that found in adults. The distribution of the cord blood lipid and lipoprotein concentrations in the sample are given, and by use of non-parametric statistics the confidence intervals for the true percentiles in the population were calculated.

KEY WORDS: Cord blood, newborns, plasmalipids, plasmalipoproteins, sex difference

Recently Kwiterovich et al (12) have presented data indicating that familial type II hyperlipoproteinaemia can be diagnosed at birth. They found that an estimate of the low density lipoprotein (LDL) concentration in cord blood rather than the total cholesterol concentration enabled them to diagnose this condition with reliability This seems reasonable as the total plasma cholesterol originates from at least two other lipoproteins than LDL. Darmady et al. (4) also concluded that estimation of cord blood total cholesterol concentration was not reliable in diagnosing familial type II hyperlipoproteinaemia.

In that ultracentrifugation analyses of plasma lipoproteins were too time consuming for routine use Kwiterovich (12) recommended the LDL estimate to be obtained by determining the LDL cholesterol concentrations. These were computed by first determining total plasma cholesterol concentration and then subtracting the high density lipoprotein (HDL) cholesterol concentration which were determined separately by precipitating the LDL and VLDL (very low density lipoprotein) with heparin and maganese This value was further corrected for VLDL cholesterol using a generally accepted formula (10)

From the examination of thirty six babies an upper cut-off limit for LDL cholesterol in normal babies of 41 mg/ml (equal to 1 06 mmol/l) was chosen representing the upper 5th percentile

The time of birth is a very convenient time

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able 1 *Percentile values of cord blood lipid and lipoprotein concentrations*
he values in bracket below each percentile value are 90% confidence values for the percentile in the population

ercentile	2 5	50	95	97.5
otal lipids, g/l	1 76 (1.60–1.88)	2.52 (2.40–.58)	3 6. (3 18–4 48)	4 04 (3.60–4.52)
otal cholesterol mmoles/l	1 45 (1.33–1 48)	2.05 (1.99–2.11)	2.85 (2.63–3.20)	2.97 (2.84–3 76)
riglycerides, mmoles/l	0.07 (0.03–0.11)	0.34 (0.28–0.36)	0.73 (0 62–0 94)	0.86 (0.73–1.01)
ree glycerol, mmoles/l	0 07 (0.03–0 08)	0.12 (0.11–0.13)	0.21 (0.19–0.24)	0.22 (0.20–0.24)
hospholipids, mmoles/l	1.26 (1 07–1 36)	1 69 (1 66–1 73)	2.16 (2.11–2.39)	2.19 (2.15–2.67)
hylomicrons, g/l	0.04 (0.00–0.05)	0 18 (0.16–0.18)	0 30 (0.28–0.34)	0.33 (0.29–0.36)
β-lipoproteins, g/l	0.48 (0.43–0.50)	0 95 (0.88–0.98)	1 63 (1 44–1 95)	1 77 (1.62–2.24)
re-β-lipoproteins, g/l	0 11 (0 00–0 19)	0 45 (0 39–0.50)	0.96 (0.90–1.07)	1 00 (0.95–1.08)
α-lipoproteins, g/l	1.23 (0 99–1.27)	2.23 (2.05–.43)	3.61 (3.25–4.36)	3 88 (3.55–4.55)

The results of these are given in Table 1 In Fig. 1 the frequency distributions are illustrated as histograms

DISCUSSION

Rather extensive studies on the cholesterol concentration in cord blood have been per formed by previous investigators (2 3 4 11 12, 13 16 17 18) Our findings are in very good agreement with the results from these studies

Darmady et al (4) found a significantly higher cholesterol concentration in girls than in boys the difference however was very small (means 81 and 76 mg/100 ml which are equal to 2.09 and 1 96 mmoles/l) The sex difference in cord blood cholesterol lev els found in the present study (medians) girls 2 11 mmoles/l (82 mg/100 ml) boys 1 96 mmoles/l (76 mg/100 ml) is in very good accordance to the results found by Darmady et al. Even if this difference at the chosen significance level, was insignificant ($p = 0.0865$) the observations support those of Darmady et al

Cord blood triglyceride and phospholipid estimations have been performed considerably more infrequently than estimations of cholesterol have been Our values of triglyceride concentrations are consistent with those of Brody et al (2) (mean 0.38 mmoles/l) and of Kwiterovich et al. (12) (mean 0 42 mmoles/l) The results of cord blood phospholipid concentrations in the present study are consider ably higher than those found by Rafstedt et al (13) (mean 1 07 mmoles/l) and somewhat higher than the results reported by Brody et al. (2) (mean 1.50 mmoles/l) The discrepancies may be explained by differences in the methods. We are not aware of any previous observations on sex differences as determined in the present study

Quantitative studies of cord blood lipoprotein concentrations in our study are compar able to those of Auerswald et al. (1) even though the lipoprotein classes are not ex actly the same and to the semiquantitative studies of Rafstedt et al. (13) and Wille et al. (19).

A better comparison however is possible with the results of Kwiterovich et al (12) who determined the cholesterol concentrations in the three major lipoprotein fractions in cord blood from 36 newborns. Using the average lipid composition in the different

for the screening of preelected groups of children for hyperlipoproteinaemia. In our opinion plasma lipoprotein concentrations can be determined precisely for clinical purposes by use of an electrophoretic technique In this paper we present upper cut-off limits for cord blood lipid and lipoprotein concentrations based on an examination of 120 newborns

MATERIAL

The study comprised 60 newborn boys and 60 newborn girls from a consecutive number of births taking place at the department of obstetrics Aalborg Sygehus Denmark The hospital serves an area of approx 150000 inhabitants. Nearly all births in this area took place in the hospital ward

In the study only newborns after a normal pregnancy were included All the mothers were free of known metabolic diseases evaluated by history physical examination and routine blood and urine analyses (e g thyreoid disease and diabetes mellitus) Children of diabetic fathers were also excluded

Diseases during pregnancy which necessitated medical treatment led to exclusion from the study Requirements for inclusion at birth were delivery at term (i e 40 weeks ±2 weeks and a birth weight of more than 2500 g) and an uncomplicated delivery (Apgar score of 10 within 2 minutes of birth)

Sampling Blood from the umbilical cord was drawn immediately after the cord was separated from the child. 10 ml of blood were collected in tubes with potassium-edetic acid (K EDTA) and stored at 4°C until analysed. All analyses were performed within 18 hours after the blood sampling.

Methods The following lipid and lipoprotein analyses were carried out. total lipids after extraction (9) total cholesterol (15) triglycerides including free glycerol (8) phospholipids (20) and lipoproteins The lipoproteins were quantitated after separation by electrophoresis in agarose gel (5) After staining the dye uptake in the different lipoprotein fractions was corrected to a value which would have appeared if all the lipid moieties in the lipoproteins were stained to the same degree per weight Thereby correcting for the differences in lipid composition of the lipoproteins The correction factors were obtained from assays with pure lipids and from knowledge of the average composition of the different plasma lipoproteins as described earlier (6 7). In this way the dye-uptake of each lipoprotein fraction was converted to actual mass concentration of lipoproteins

Statistics Reference values normal range" and cut-off limits were based on nonparametric estimates as described by Reed et al (14) In that a sample size of 120 permits an estimate of 90% confidence intervals for the 2.5 percentile value in the population which could not be obtained at a smaller number a sample size on 120 was chosen.[1] We use the notations of Reed et al (14) where the rank order for confidence intervals for the 2.5 percentile and 97.5 percentile are tabulated as a function of *n*

Confidence intervals for other percentiles were calculated from the following equation

$$\sum_{j=a}^{b-1} \binom{n}{j} (p)^j \quad (1-p)^{n-j} \geq 0\ 90 \qquad \text{(I)}$$

where a and b are the rank orders for the test values x_a x_b which limit the 90% confidence interval for the p percentile in the population.

In statistical comparisons of medians the Mann-Whitney rank-sum test was used in that Gaussian distributed rank-sums were assumed.

RESULTS

Chosing a probability level of $p_{2a} \leq 0\ 05$ the only significant sex difference observed was a higher phospholipid concentration in females median 1 75 mmoles/l (range 1 26–2 39 mmoles/l) than in males medial 1 66 mmoles/l (range 1 07–2 18 mmoles/l) the p value was 0 0443

Differences with probabilities due to chance of approx double the magnitude of that of phospholipids were found in cholesterol concentration males median 1 96 mmoles/l (range 1 33–3 76 mmoles/l) females median 2 11 mmoles/l (range 1 42–2 87 mmoles/l) (p=0 0865) and in alpha lipoproteins males median 2 12 g/l (range 0 99–4.55 g/l) females median 2 50 g/l (range 1 26–3 75 g/l) (p=0 0773) No other lipid or lipoprotein parameter showed any sex differences at a probability level below 0 10

As the only sex difference with a probability level below 0 05 was the plasma phospholipid concentration and as this difference had a very low *clinical* significance (difference between medians at 0 09 mmoles/l) it seemed justified to evaluate normal limits from the values of all 120 subjects

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[1] Using formula (I) we have calculated the critical sample sizes allowing estimation of confidence intervals around different percentiles. We obtain the same values as given by Reed et al (14) except that we find that a sample size of 119 is large enough to estimate the 90% confidence interval for the 2.5 percentile value.

lipoprotein classes as indicated in a previous study (6) lipoprotein concentration in the present study can be expressed in terms of lipoprotein-cholesterol By use of a mean molecular weight of cholesterol esters of 671 we compute a mean α-lipoprotein cholesterol concentration of 0.30 g/l β-lipoprotein cholesterol of 0.29 g/l and pre-β-lipoprotein cholesterol of 0 08 g/l The corresponding average values found by Kwiterovich et al (12) are 0.37 g/l, 0.31 g/l and 0 06 g/l respectively These values are nearly identical. Kwiterovich et al. stress in their study that the use of the formula LDL cholesterol=Total cholesterol−(TG/5+HDL cholesterol) for cord blood, needs confirmation as it assumes a composition of lipoproteins in newborns equal to that found in adults.

The results of Kwiterovich et al. (12) are based mainly on ultracentrifugation studies. They however advocate a more simple approach for LDL-estimation as mentioned earlier In this a presumption of equality in lipoprotein composition in adults and newborns is necessary and this equality has yet to be proved. Our results expressed in terms of lipoprotein cholesterol are based on the same assumption The similarity of the results in the two studies tends to verify this hypothesis Their cut-off limit for LDL cholesterol concentration representing the upper 5th percentile as 0 41 g/l is also directly comparable to our results, as the 95 percentile for β-lipoproteins of 1 63 g/l equals a β-lipoprotein cholesterol concentration of 0 48 g/l. The confidence limits of a given percentile are due to the larger sample size in this study (120 compared to 36) correspondingly more narrow

As hyperlipoproteinaemia can often be ameliorated during infancy by diet, the importance of early detection is obvious The moment of birth is a convenient time for the evaluation of such a diagnosis as blood sampling in adequate amount is easy to obtain and dietary treatment may be initiated before consideration of its palatability becomes a precondition The usefulness of this sort of lipid and lipoprotein analyses of cord blood except for preselected high risk groups have yet to be evaluated

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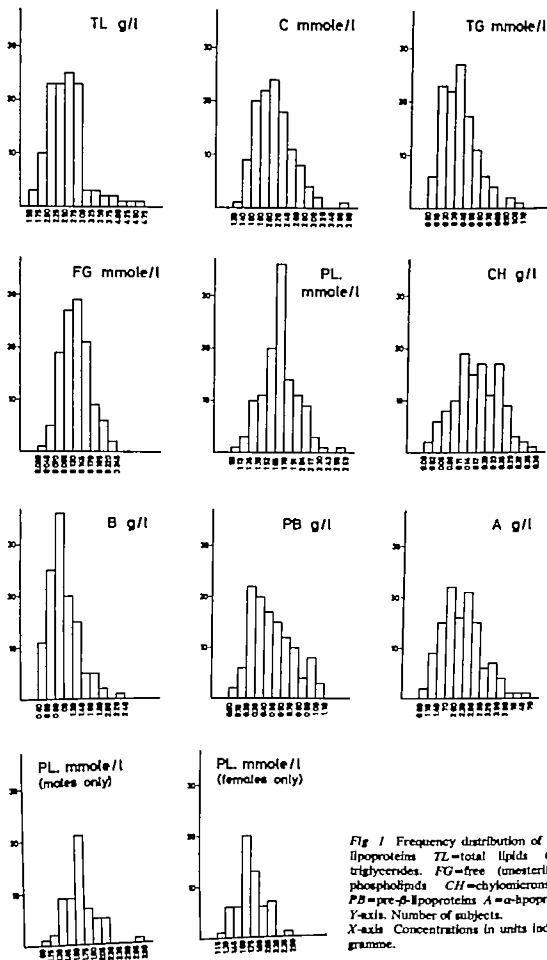


Fig 1 Frequency distribution of cord blood lipids and lipoproteins *TL*=total lipids *C*=cholesterol. *TG*=triglycerides. *FG*=free (unesterified) glycerol *PL*=phospholipids *CH*=chylomicrons. *B*=β-lipoproteins *PB*=pre-β-lipoproteins *A*=α-lipoproteins.
Y-axis. Number of subjects.
X-axis Concentrations in units indicated in each histogramme.

Acta Pædiatr Scand 63 437–442, 1974

PYCNODYSOSTOSIS

Six Cases with New Symptoms and an Autopsy

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ABSTRACT Lykkegaard Nielsen, E. (Department of Paediatrics, Sundby Hospital, Copenhagen, Denmark.) Pycnodysostosis. Six cases with new symptoms and an autopsy. Acta Paediatr Scand, 63: 437, 1974.—Six patients with pycnodysostosis, all of whom had characteristic case-histories, are presented. Five of the patients had symptoms which have not hitherto been recognized in this condition. They all had varying degrees of respiratory distress, tendency to vomit and long soft palates. One of the patients died at the age of 21 months on account of vomiting and pulmonary aspiration. Autopsy revealed normal conditions in the lungs. The vital prognosis is usually stated to be good in this disease but this study shows that during the first years of life it is possibly poorer. Occurrence of progressive acroosteolysis in pycnodysostosis has been discussed previously. In one of the patients in this material slight increase in the bony substance in the distal phalanges of the fingers was observed on repeated radiografic examination while in another both break-down and new bone formation were observed in the processus unguicularus. In a third patient cyst-like defects in some of the ungual tufts of the fingers were present. These conditions are thus incompletely elucidated at present.

KEY WORDS: Pycnodysostosis

Pycnodysostosis can be defined as a syndrome consisting of the following characteristics: dwarfism, generalized osteosclerosis, open fontanelles, failure of closure of cranial sutures, blunt mandibular angle, defective terminal phalanges of hands and feet, a tendency to bone fractures, and normal intelligence.

The syndrome was first described by Montenary (12) in 1923. It was recognized as a distinct entity in 1962 by Maroteaux & Lamy (9) who separated the disease from osteopetrosis, marble bones (1, 7, 10), and proposed the name of pycnodysostosis. In the same year Andrén et al. (2) described the syndrome as osteopetrosis acro-osteolytica, but this name was not generally accepted. A total of 79 cases has been reported, and the disease has been reviewed by Elmore (5) in 1967. The most important differential diagnoses are osteopetrosis, cleidocranial dysostosis, and osteogenesis imperfecta as shown in Table 1. A single case of pycnodysostosis with haemolytic anaemia and hepatosplenomegaly has been reported (8).

The present study will describe some new symptoms and morfological deviations in this disease. Also the result of an autopsy of one case will be given, as no post-mortem examinations hitherto have been published.

CASE REPORTS

The six patients in the present investigation had all the characteristic symptoms of pycnodysostosis, as described in the introduction of this article. Therefore in the following case reports no further documentation

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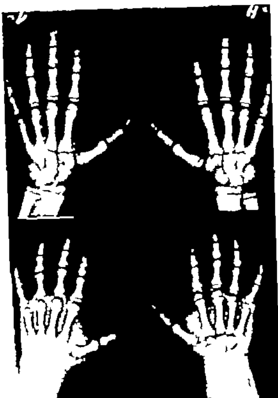


Fig 1 Case 4 Hands at the ages of six (bottom picture) and nine years (top picture) respectively See text.

Case 5 Boy brother of case 6. For the whole of his life the patient has had a tendency to snoring and vomiting, this is accentuated by colds. Marked tendency to sweating.

At the age of 36 years the palate was highly grooved, fused but with cleft uvula. The soft palate was long and almost reached the root of the tongue. Radiologically generalized osteosclerosis was seen with open fontanelles and cranial sutures (Fig. 2). The portion between the base and the unguiculate process of all terminal phalanges on the right hand was missing. On the right foot pseudarthroses were seen on the second and the third metatarsal bones and the terminal phalanges were stumpy and tapering without unguiculate processes (Fig. 3) At the age of 13 years this patient (and his sister case 6) were examined by Sjölin (13). The clinical findings were the same as was the X-ray picture of the skull (Fig 7) The X-ray picture of the right hand was very much like that from the age of 36 years, although the unguiculate processes of the first and the fifth finger were missing, and the base of all terminal phalanges were less developed (Fig 4).

Case 6 Girl, sister of case 5 From three to seven months old constantly admitted to hospital because of febrile episodes and periods of respiratory insufficiency Catarrhal for long periods with snoring respiration during sleep At the ages of ten and fourteen years pneumonia. At the age of fourteen years menarche followed by rather regular menstruations. Catarrhal for a long period two or three times a year with snoring and foreign body sensation in the throat. Pronounced sweating.

At the age of 20 years the hard palate was highly grooved, the soft palate rather long, but not as long as that of her brother Hypermobility of the joints of the hands was present.

DISCUSSION

Respiratory insufficiency a tendency to vomit and a long soft palate seem to be unnoticed symptoms in pycnodysostosis until now All six patients in this investigation displayed one or more of these signs to some degree. Cases 1 and 2 had pronounced snoring, stridulous respiration frequent respiratory tract infections and a pronounced tendency to regurgitations and vomiting. Case 1 died from this. Case 3 at the age of three developed snoring, but this symptom disappeared after adenoidectomy Case 4 in her first year of life had a tendency to vomiting after almost every meal Case 5 for the whole of his life has had a tendency to snoring and vomiting, especially when catarrhal. In early infancy for long periods case 6 was admitted to hospital because of respiratory insufficiency and respiratory tract infections with snoring respiration Laryngoscopy in all six patients was normal A uniform sign in cases 2, 4 5 and 6 was a long soft palate which almost reached the root of the tongue This can explain both snoring, and possibly stridulous respiration the tendency to vomit, and the fact that the symptoms became worse when respiratory tract infections complicated the disease The length of the soft palate in case 1 was not described particularly in the records but he had the same snoring, stridulous respiration as his brother Case 3 had a normal soft palate in accordance with the fact that she had no symptoms from the throat except for adenoids at the age of three years. Thus this investigation shows that if a patient with pycnodysostosis has a long soft palate is vomiting and snoring, and has a

Table 1 *Differential diagnostic characteristics between pycnodysostosis osteopetrosis cleidocranial dysostosis and osteogenesis imperfecta*

	Pycnodysostosis	Osteopetrosis	Cleidocranial dysostosis	Osteogenesis imperfecta
Nanism	+	−/(+)	+	+
Autosomal inheritance	Recessive	Recessive*	Dominant	Dominant
Generalized osteosclerosis	+	+	−	−
Open fontanelles and sutures	+	−	+	−
Tapering distal phalanges	+	−	−/(+)	−
Blunt mandibular angle	+ ≤180°	−	−/(+)	−
Clavicular anomalies	Hypoplasy of lateral ends	−	Hypoplasy or total aplasy	−
Wormian bones	+	−	+	+
Bossing of frontal bones	+	−	+	+
Hypoplasy of facial bones	+	−	+	−
Tendency to bone fractures	+	+	−	+
Presence of medullar cavity in tubular bones	+	−	+	+
Anaemia	−	+	−	−
Hepatosplenomegaly	−	+	−	−
Compression of cranial nerves	−	+	−	−
Ossification time	Normal	Normal	Delayed	Normal
Blue sclerae	−	−	−	+
Prognosis quo ad vitam	?	Bad	Good	Bad

* The inheritance of a mild type is autosomal dominant

for the correctness of the diagnoses will be given but only new symptoms will be mentioned The ages at which the patients are examined are stated in each case

Case 1 Boy brother of case 2 The parents are first cousins Since birth failure to thrive because of constant regurgitations and vomiting after nearly all meals Almost constantly catarrhal with snoring, stridulous respiration Admitted to hospital 10 14 and 20 months old because of respiratory insufficiency

At the age of 20 months the palate was highly arched with a deeply grooved but fused median raphe No sign of vascular ring. The bone age was approximately 9–12 months.

Twenty-one months old the patient was found dead in bed after vomiting with aspiration to the lungs Autopsy showed food obstruction in the respiratory tract. The hard palate was highly grooved the soft palate normal although the length of it was not mentioned in the record The larynx trachea bronchi liver and spleen were normal A fracture of the right clavicle was found On the inside of the frontal and occipital bones numerous very thin exostoses of 1–10 mm were seen The orbital vault was slightly thickened and shortened Histological findings were normal especially regarding the liver the spleen a transverse section of the sagittal suture and adjoining bone a hyperostosis from the frontal bone and a longitudinal section of the clavicle

Case 2 Boy brother of case 1 During the first three months almost constantly catarrhal with snoring, stridulous respiration and abundant formation of secretion in the upper airways Eating was difficult because of regurgitation and a tendency to vomit. Admitted to hospital 3 8 and 10 months old because of respiratory insufficiency and pronounced nasopharyngitis. The first admittance to hospital lasted more than four months. The patient was artificially ventilated for three days, and a persisting splenomegaly developed.

At the age of 12 months the hard palate was highly grooved the soft palate was long and almost reached the root of the tongue

Case 3 Girl At the age of three years a snoring respiration arose which disappeared after adenoidectomy three years later

At the age of six years the soft and the hard palate were normal Radiologically on the left hand short phalanges and metacarpal bones with cyst-like configurations in the ungual tufts of the first and the fifth finger were seen A similar cyst like defect was found in the first toe of the left foot

Case 4 Girl. During her first year tendency to vomit after almost all meals. This disappeared slowly during the next years No snoring. Menarche 14 years old and thereafter rather regular menstruations.

At the age of 23 years the soft palate was long and almost reached the root of the tongue Hypermobility of joints of hands and elbows was present The X-ray pictures of the hands at the ages of six and nine years respectively (Fig. 1) showed that a cyst-like defect had appeared in the ungual tuft of the right fifth finger while on the left first finger a similar defect had disappeared. At both ages partly missing ungual tufts in some of the other fingers were found At the age of six years X-ray picture of the feet showed tapering of the phalanges, and three years later the same changes were seen

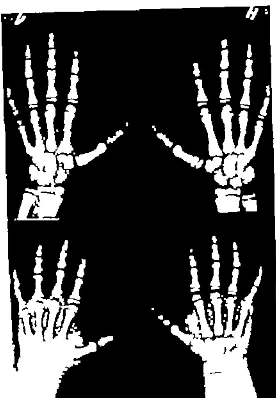


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Fig 2 Case 5 Top picture Skull at the ages of 13 (left) and 36 years (right) Bottom picture Skull at the age of 36 years Fontanelles and cranial sutures are open The form of the skull and the pronounced osteosclerosis seem unchanged during this period of 23 years.

stridulous respiration this may indicate a worse prognosis because of risk of aspiration to the lungs or respiratory insufficiency Otherwise until now the prognosis has been considered generally good Previously the death of only one patient has been reported (4) a five-months-old African boy who died from a respiratory tract infection Autopsy however was not performed

The tapering or fragmentation of the unguiculate processes has interested several authors (2 3 6) Kaju et al (6) proved that a patient in the course of two years developed a progressive acro-osteolysis of the terminal phalanges of the first and second fingers of one hand In the present study the X-ray pictures of case 4 (Fig. 1) have been compared at the ages of six and nine years During this period a cyst-like defect appeared in the unguiculate process of the fifth finger of the right hand while on the thumb of the left hand a similar defect in the unguiculate process disappeared In case 5 by comparing the X-ray pictures at the ages of 13 and 36 years (Figs 3 and 4) a slight augmentation of the bone substance of the terminal phalanges has been demonstrated At the same ages X-ray pictures of the skull and the degree of osteosclerosis were constant (Fig 2) In case 3 cyst like configurations in the ungual tufts of the first and fifth fingers were seen Thus these investigations state that in some cases both bone destruction and bone formation take place and this can be registered within a few years

Among the patients described in this study

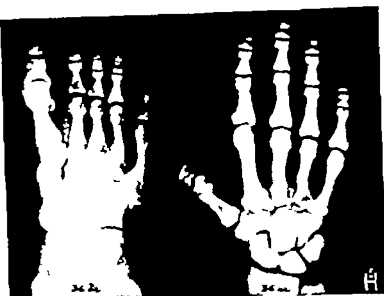


Fig 3 Case 5 Right hand and foot at the age of 36 years. See text.



Fig 4 Case 5 Right hand at the age of 13 years. See

two were adult women Since the age of 14 years they have had rather regular menstruations none of them had children In the literature a woman with pycnodysostosis who has given birth to two healthy daughters is reported (3), and a man with pycnodysostosis married to a healthy woman and with a healthy daughter aged 15 is described (11)

Autopsy of case 1 showed normal conditions in the larynx the bronchi and the lungs, and thus gave no explanation to the patient s stridulous respiration As to the numerous thin exostoses on the inside of the frontal and occipital bones the reason as well as the significance of these are not clear but except for this finding the autopsy revealed nothing unexpected.

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LETTER TO THE EDITOR

FREQUENCY OF SOME METABOLIC DISORDERS IN POLAND

In Poland a screening programme for the detection of certain metabolic disorders was introduced 6 years ago The use of this screening programme is greatly facilitated by the fact that nearly all deliveries take place in hospitals or labour wards where the new borns stay for 3–7 days For this reason it is possible to carry out screening tests in cooperation with the departments for new borns. Investigations are done at the National Research Institute for Mother and Child The system of investigations includes the follow ing blood screening tests

(1) Guthrie bacterial inhibition assay for PKU

(2) Guthrie bacterial inhibition assay for tyrosine

(3) Guthrie bacterial inhibition assay for histidine

(4) Beutler and Baluda fluorescent test for galactosemia.

All tests are done on blood specimens obtained from newborns between 4–7 days of age Beutler and Baluda tests are done on a filter paper modification Results of testing and frequency of the specific conditions are as follows.

Guthrie testing for PKU

Total newborns screened	No. of cases with persistent phenylalaninemia	No. of phenylalaninemias non PKU cases initially tested with low phe diet	No of atypical PKU cases	No of typical PKU cases
793 838	115	6	8	101

From the above results it can be concluded that the frequency of all types of persistent phenylalaninemia in the Polish population varies around 1 7000 Frequency of classical PKU is around 1 8000 Frequency of PKU variants remains at 1 50000

Guthrie bacterial inhibition assay for tyrosine

Total newborns tested	Result of Guthrie test for tyrosine			
	N of presumptive positive results	Cases with increased tyr level in control	No. of cases with full normalisation	Not controlled
109 112	58	2	43	10

Guthrie bacterial inhibition assay for histidine

No of newborn infants screened	Results of Guthrie test for histidine			
	No of presumptive positive results	No of cases with total normalisation	No of cases with increased hist level in control tests	No of not controlled cases
109 338	191	138	6	45

No case of histidinemia was found

Beutler and Baluda test for galactosemia
The present stage of the study on early detection of galactosemia is the following

Total number of Beutler and Baluda tests performed 216 178 number of abnormal results 1 124 (0 52% of all tests performed) number of confirmed galactosemia cases 15 number of detected galactosemic heterozygotes 52 number of detected galactosemic heterozygotes (Duarte Variant) 6

The frequency of galactosemia in the Polish newborn population is 1 14 412

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LETTER TO THE EDITOR

Sir

In their article "Urinary Phenylalanine Excretion in Hyperphenylalaninaemic Children" (*Acta Paediat Scand* 62 333 1973) F Güttler & F Rosleff present data which they state do not support our suggestion that hyperphenylalaninemic patients are protected by their increased urinary excretion of phenylalanine

They rightly point out that our suggestion was merely a hypothesis. Indeed we hoped it would promote further research such as that performed by Güttler and Rosleff However I would contend that the hypothesis is not completely excluded by their study in which they gave a highly unphysiologic dose of phenylalanine In addition, their phenylketonuric children were on low phenylalanine milk substitutes which both we and others (Clayton, B E. Francis D E M Shepherd J & Wolff O K. *Arch Dis Child* 45 640 1970 and Clayton B E Heeley A F & Heely M *Brit J Nutr* 24 573 1970) have shown to cause an amino aciduria, whereas the comparison we made was between phenylketonuric children on normal diet and hyperphenylalaninaemic children on normal diet. In the light of this it is of great interest to note that the hyperphenylalaninaemic children of Güttler and Rosleff before loading, show a higher phenylalanine creatinine ratio than the phenylketonuric children when the serum concentrations of phenylalanine in both groups were identical.

Thus, while the article of Güttler & Rosleff does not confirm our hypothesis it has not disproved it.

D R Lines

The Editor has asked dr Güttler to comment on the remarks made by dr Lines

Sir

In their article (*J Pediat* 78 474 1971) Lines & Waisman examined the hypothesis that hyperphenylalaninemic individuals could possibly be phenylketonuric but partially protected by a renal tubular transport defect which caused them to lose phenylalanine (phe) in the urine Their study included. 11 phenylketonuric patients off diets with a mean serum phe of 33 9 mg/100 ml and a mean 24 h excretion of 539 mg phe per g creatinine seven hyperphenylalaninemic individuals with a mean serum phe of 16 7 mg/100 ml and a mean excretion of 409 mg phe per g creatinine and seven phenylketonuric patients on diets with a mean serum phe of 11 4 mg/100 ml and a mean excretion of 241 mg phe per g creatinine Unfortunately no details are given about 95% confidence limits ranges or standard deviations on these data. However according to the authors phenylalanine is not among the amino acids which the hyperphenylalaninemic individuals excreted at significantly greater amounts than phenylketonuric patients off diets Our data (Güttler F & Rosleff F *Acta Paediat Scand* 62 333 1973) are in agreement with this observation. The median excretion before loading with phe of five hyperphenylalaninemic individuals was 390 µmol phe per g creatinine (range 280–1 014) and the median excretion of eight fasting phenylketonuric patients on diet before loading was 285 µmol phe per g creatinine (range 69–1 151) As the number of observations does not allow any assumption

Guthrie bacterial inhibition assay for histidine

No of newborn infants screened	Results of Guthrie test for histidine			
	No of presumptive positive results	No of cases with total normalisation	No of cases with increased hist level in control tests	No of not controlled cases
109 338	191	138	6	45

No case of histidinemia was found

Beutler and Baluda test for galactosemia
The present stage of the study on early detection of galactosemia is the following

Total number of Beutler and Baluda tests performed 216 178 number of abnormal results 1 124 (0 52% of all tests performed) number of confirmed galactosemia cases 15 number of detected galactosemic heterozygotes 52 number of detected galactosemic heterozygotes (Duarte Variant) 6

The frequency of galactosemia in the Polish newborn population is 1 14 412

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Acta Pædiatr Scand 63 447–449 1974

CASE REPORT

A LIVEBORN TRIPLOID INFANT

P HENRIKSSON L. HÅKANSSON and B. SANDAHL

From the Department of Paediatrics Allmänna sjukhuset Malmö University of Lund and the Institute of Embryology University of Lund S eden

ABSTRACT Henriksson, P., Håkansson, L. and Sandahl, B. (Department of Paediatrics, Allmänna sjukhuset, Malmö, University of Lund, and the Institute of Embryology University of Lund, Sweden). A liveborn triploid infant. Acta Paediatr Scand, 63: 446, 1974.—A liveborn triploid infant, a boy born after 34 weeks' gestation, is described and short review of earlier cases is given. The phenotype agreed with that connected with triploid infants, low birth weight, low viability adrenal hypoplasia and syndactyly of third and fourth digits. Cytogenetic studies made on cultivated leucocytes also including autoradiographic analyses showed triploid number of morphologically normal chromosomes. The chromosome constitution was 69/XXY No signs of mosaicism were found.

KEY WORDS: Chromosome aberrations, complete triploidy congenital malformations

Triploidy is relatively common in man and it has been estimated that at least 1% of all human conceptuses are triploid (2) The greater part of these are aborted but a few cases of full-term or near-term infants have been reported A recent survey of full-term infants published till 1972 has been given by Zergollern et al (10). Since then 3 more cases have been re ported (4 7 9)

Mosaicism involving a triploid cell-line is relatively uncommon in spontaneous abortuses (?) while a diploid/triploid mosaicism has been reported in several of the full-term children (1 3 5)

In the present paper we report on a liveborn child with pure triploidy

Case History

The patient a boy was the first child of healthy unrelated parents The mother was 25 years old at delivery The father was 27 years old The pregnancy was normal during the first 4 weeks, after which the mother suffered from therapy-resistant hypertension Neither albuminuria nor oedema appeared. At the 28th week the mother had a temporary attack of itching with increased transaminase activities in serum (GOT 63 GPT 46, upper normal limits 38 and 30 respectively). The bilirubin concentration in serum was normal. A normal delivery oc curred in the 34th week of gestation. The amniotic fluid was yellow and clear The placenta was large (1400 g) and there were three vessels in the umbilical cord. No histological examination of the placenta was made

The boy weighed 1730 g. Apgar score 5 at 1 minute The patient was extremely hypotonic and the neonatal

Table 1 *Comparison of some of the common features of 13 reported triploid new borns*

Features	*Present*	*Not present*	*Not registered*
Low birth weight (≤2500 g)	13	0	0
Low viability (≤24 hrs)	9	3	1
Simian crease	9	3	1
Syndactyly third and fourth digits	8	5	0
Microphthalmos	6	4	3
Coloboma	8	4	1
CNS anomalies	6	3	4
Genital anomalies (cryptorchidism excluded)	8	4	1
Adrenal hypoplasia	7	2	4
Large placenta	7	0	6

about the distribution of the data e g a Gaussian distribution a nonparametric test must be used The Wilcoxon rank sum test shows that our data do not justify rejection of the hypothesis that hyperphenylalaninemic individuals and phenylketonurics excrete phenylalanine to the same extent even at a 0 10 level of significance

Serum phe levels of untreated phenyl ketonurics are usually above 1250 μmol/l (20 mg/100 ml) which might be characterized as a physiological level of serum phe in these patients Even at levels of this magnitude renal reabsorption of phe is almost complete (Brodehl J Gellissen K & Hagge W *Monatsschr Kinderheilk* *116* 305 1968 Rosenberg L. E & Scriver C R *in* P K Bondy & L E Rosenberg (eds) *Diseases of metabolism* W B Saunders Co Phila delphia 1969 6th edition p 478) If hyper phenylalaninemic individuals are phenyl ketonurics protected by an increased urinary excretion of phe e g by an impaired renal reabsorption of this amino acid this would be easy to demonstrate by raising the serum phe of hyperphenylalaninemics to that of untreated phenylketonurics This test how ever revealed that hyperphenylalaninemic individuals excreted phenylalanine in lesser quantities during the following 24 hours than patients with phenylketonuria in spite of the fact that serum phe of hyperphenylalanin-emic individuals returned to preloading levels (285 μmol/l or 4 7 mg/100 ml) within the same period of time Especially during the first six hours after phe loading hyper phenylalaninemic individuals excreted sig-nificantly less phe than did phenylketonurics who had obtained their first meal of the day containing Albumaid less than one hour be fore the termination of this period of urine collection Clayton et al (Clayton, B E. Heely A F & Heely M *Brit J Nutr* *24* 573 1970) observed an increased urinary ex cretion of alpha-amino nitrogen in the urine samples collected four hours after the inges-tion but not two hours after the ingestion of Albumaid Thus hyperaminoaciduria due to the phenylalanine free acid-hydrolysate protein preparation used in our study can not explain the increased phe excretion of phenylketonuric patients within the first six hours period of urine collection. Further more Clayton et al assume that the observed mean increase in urinary alpha-amino nitrogen following the ingestion of acid-hydrolysates of casein might be accounted for by the presence of D-amino acids in the diet.

F Guttler

Acta Pædiatr Scand 63 447–449 1974

CASE REPORT

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F Güttler

The heart was somewhat enlarged with a prominent left ventricle. Foramen ovale was wide open Otherwise the heart was normal. The lung vessels were normal Some parts of the lungs were atelectatic and the capillaries contained numerous erythropoietic cells. The thymus was atrophic Except for an omphalocele the topography of the abdominal viscera was normal The bile ducts were small but had a normal topography The gall bladder was hypoplastic. The liver was remarkably large The kidneys were low set. The right kidney had a few small cysts in its lower pool. The ureters and the urinary bladder were normal. The adrenals were markedly hypoplastic with a thin cortex. The testes were large and still intra-abdominal They showed hyperplasia of the interstitial cells. The histological appearance of the kidneys, liver and heart was normal.

Cytogenetic findings

Leucocytes were cultured according to a micromethod. Two drops of whole blood are added to 1.5 ml of culture medium (70% Parker 199 30% human blood donor serum inactivated at 56°C for 30 min and 5.5 IU of heparin per ml). Otherwise the cytogenetic procedures were carried out according to Källén & Levan (6).

Chromosomes were counted in 100 cells. 92 cells had 69 chromosomes (Fig 1) Eight cells had a hypo-triploid number of chromosomes. This is most likely an artifact due to disruption of the cells when making the preparations. No cell with a diploid number of chromosomes was found Fifteen cells were carefully analysed all of them had three haploid sets of morphologically normal autosomes. The sex chromosomes constitution was XXY

Unfortunately there was no possibility of analysing the karyotype of other tissues as the infant died.

An autoradiographic study of the late labelling pattern was performed using the method described by Schmid (8). Ten cells were studied. The labelling of the three homologous chromosomes seemed to be similar and no late labelling X-chromosome was found

DISCUSSION

Up to the present, twelve liveborn children with presumptively pure triploidy have been described. This appears to be an additional such case since no diploid cells were found in cultures from peripheral blood Unfortunately it was not possible to analyse any other tissues However in the reported cases of mosaicism the two kinds of cells were consistently found in the lymphocyte cultures while only one type of cells was found in the skin cultures

A fairly clear-cut clinical picture of a triploid syndrome can now be outlined The most frequent signs of this syndrome are presented in Table 1

The present case is in good agreement with the earlier reported cases

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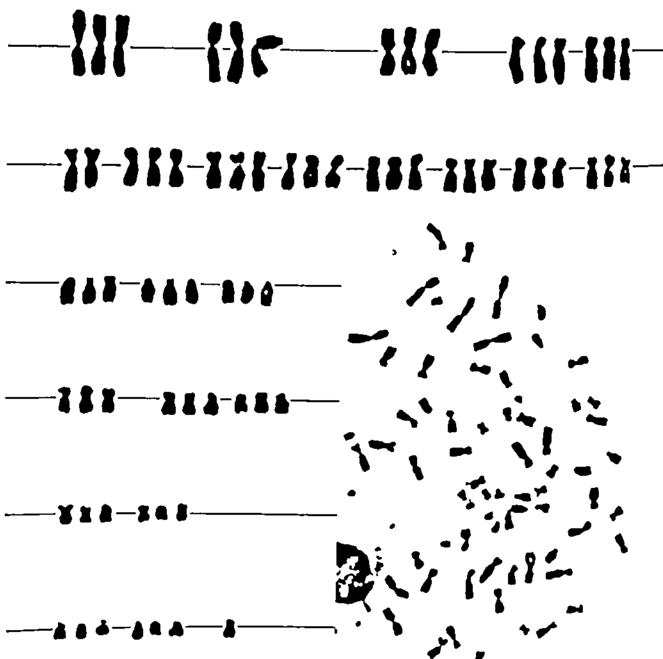


Fig 1 Karyotype of the patient

reflexes were absent. The immature appearance corresponded to the 34th week of gestation

The following malformations were observed. Omphalocele total syndactylia of the 3rd and 4th fingers of the left hand and partial syndactylia of the corresponding fingers of the right hand, and partial syndactylia of the 2nd 3rd and 4th toes bilaterally External genitalia were normal for the age except for bilateral retentio testis

The patient developed a grave respiratory insufficiency and died 9 hours after delivery

Autopsy

A large cephalic haematoma was found The leptomeninges showed some small petechial bleedings The basal arteries of the brain were normal The brain had a normal configuration corresponding to gestational age



Fig. 2. Actinomycotic colonies, in the centre deeply stained by hematoxylin. Toward the periphery eosinophil hyphae extending in a non-branching manner. Numerous inflammatory cells surrounding the colonies. Hematoxylin-eosin, ×300.



Fig. 3. The border of an actinomycotic colony with characteristic rim of polymorphonuclear leukocytes adhering to the periphery of the colony. Hematoxylin-eosin, ×300.

Case Report

J. R., a boy born November 11, 1965, second child of healthy parents, was first admitted to the Department of Paediatrics, University Hospital of Umeå in 1972, because of a 3 month long "feeling of discomfort" in the perineal region. Neonatal period and general development was normal. Immunization against tuberculosis and smallpox had been performed with positive results. There was no history of infection susceptibility. The examination revealed a fluctuant, perianally located tumour, 2×1 cm, slightly to the left. The tumour was not attached to the skin or to the underlying structures. There had been no trauma, infection or insect bite.

Haemoglobin concentration was 12.0 g/100 ml, sedimentation rate 5 mm/h and the differential count of the leukocytes was normal. Roentgenographic examinations of the lungs, the stomach, small and large intestines were normal. A benign tumour, a cyst, a hygroma or a chronic perianal abscess was suspected. After an observation time of a couple of months without any notable change in the size of the tumour, an extirpation was performed at the Department of Surgery. It was found that the tumour consisted of granulation tissue and debris, partly encapsulated. The debris was greyish, consisting partly of granules. No fistula to the rectal wall could be found either at the exploration or at a conventional operative proctoscopy. The granulation tissues were curetted.

The histopathological examination revealed colonies of actinomycetes (Fig. 1) in a well vascularized non-specific granulation tissue with abscess formation. The colonies, measuring mm in diameter or less, were gram-positive and stained deeply basophilic with hematoxylin. On the periphery, radially oriented, eosinophilic hyphae, mostly non-branching, extended from the basophilic centre (Fig. 2) just a short distance into the surrounding granulation tissue. Adjacent to the fungus colony a rim of polymorphonuclear leukocytes was seen (Fig. 3).

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CASE REPORT

LOCAL PERINEAL ACTINOMYCOSIS

LENNART DOMELLÖF SUNE ERIKSSON GÖSTA SAMUELSON and BO SÖNNE

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ABSTRACT Domellöf L. Eriksson S. Samuelson G. and Sönne B. (Departments of Pathology Paediatrics and Surgery University Hospital, Umeå Sweden) Local perineal actinomycosis. Acta Paediatr Scand 63: 450 1974.—A case of perineal actinomycosis in a 7-year-old child is reported. A localized tumour was excised and actinomycetes was demonstrated histologically The etiology therapy and prognosis are discussed The importance of microscopical examination of infections in the ano-rectal area is emphasized

KEY WORDS: Actinomycosis, perineal diseases

Actinomycosis is defined as a chronic granulomatous infection caused by the ray fungus *Actinomyces israelii* It has a wide geographical distribution and is a generally non pathogenic anaerobic non-acid fast organism When present it appears to lead a saprophytic existence in the respiratory and gastro-intestinal tracts It may however become pathogenic being the most common of the highly fatal mycoses The disease may develop in any part of the body starting with a local infection process spread by direct extension rather than by lymphogenous or haematogenous invasion It is a relatively uncommon disease in adults and only a few cases have been found in children (1) The present report is given in order to present some of the diagnostic problems of actinomycosis in a child with an unusual localisation

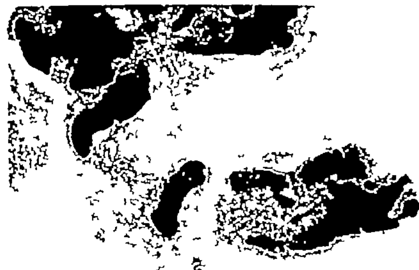


Fig 1 Section from contents of perineal cyst Conglomerate of actinomycotic colonies floating in pus. Hematoxylin-eosin, ×50

Acta Pædiatr Scand 63 453–456 1974

CASE REPORT

POST NATAL INFARCTION OF THE RIGHT LOBE OF THE LIVER

F HARTVEIT B MÆHLE and S. BÖRSTING

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ABSTRACT Hartveit, F., Mæhle, B. and Börsting, S (The Gade Institute, Department of Pathology and The Department of Paediatrics, University of Bergen, Norway). Post natal infarction of the right lobe of the liver Acta Paediatr Scand 63: 453 1974.—A case of hypoplasia of the hepatic artery with infarction of the right lobe of the liver is reported. The part played by the arterial as well as the venous blood supply to the liver cells in the immediate post-natal period is discussed. It is stressed that infarcts of the liver may occur in the presence of arterial insufficiency whether venous occlusion is present or not.

KEY WORDS: Liver infarction, neonate

Infarction of the liver is rare and there has been much discussion as to its causes (10) It may follow ligation of the hepatic artery or its branches (7) or obstruction due for example to periarteritis nodosa or endarteritis One case due to hypoplasia of the hepatic artery in a newborn infant is on record (13) Infarction has been recorded when only portal vein radicals were occluded (10) and there is one re port of infarction in a newborn infant without vascular occlusion in the absence of recog nized hypoplasia of the hepatic artery (10) Infarcts in the absence of vascular occlusion have also been reported in connection with cardio-vascular disease or surgical shock (1? 10)

The part played by the venous blood supply in the production of degenerative changes in the liver seen in the neonatal period (4 3) and the association of hepatic infarction with catheterization of the umbilical vein (9) have been stressed previously The present case is reported as it highlights the role of the hepatic artery in the neonatal period.

CASE REPORT

The child, a boy weight 3800 g, length 54 cm, was lifeless on delivery following an uneventful pregnancy and normal delivery The cord was wound 3 times around its neck. Following resuscitation, which included 10 mEq hypertonic sodium bicarbonate and 20 ml 20% glucose via the umbilical vein, heart action and spontaneous respiration were established. He was given oxygen and a catheter was inserted into the umbilical vein. Slight acidosis was corrected with isotonic sodium bicarbonate. Later infusion of isotonic glucose was given. Generalized convulsions set in, but were fairly well controlled with Valium. Respiratory distress continued, the liver increased in size and periods of apnoea occurred and increased in duration until the child died 39 hours old.

At *autopsy* (0.988/72) there was no jaundice or cutaneous haemorrhage No external malformations were present.

The *liver* weighed 150 g The right lobe was yellowish-grey and soft with a clear line of demarcation (Fig 1) from the left physiological lobe which was reddish-brown and firm Dissection of the *hepatic artery* showed that the vessel was hypoplastic, the lumen of the main trunk being just visible with the naked eye It divided into three main branches before entering the liver The left hepatic arose from the main trunk just distal to the gastro-duodenal artery Distal to this again the trunk divided into 2, a right hepatic that divided into 3 minor branches before entering the liver parenchyma and a middle hepatic that also divided into several

The postoperative course was uneventful Penicillin was administered for 2 months No signs of local recurrence or dissemination have been found

DISCUSSION

Actinomycosis is generally reported as a rare disease in man (1 3) but there are probably many undiagnosed cases From the Mayo Clinic Putman and co-workers report an average of 3–4 cases a year (1) They collected 122 cases of actinomycosis during a period of 35 years Four probably adults of the 122 cases had ano-rectal involvement generally with a long-standing history of fistula-in-ano In large materials 50% of the clinical manifestations are found in the cervico-facial region 15% in the thoracic and 20–30% in the abdominal region (1) It may simulate tumour non specific infection tuberculosis or other granulomatous disease Samenius in 1965 published five adult cases of ano-rectal actinomycosis collected over a period of 15 years (2) No case of ano-rectal actinomycosis has been described in childhood

The most important single sign of actinomycosis is the detection of sulphur granules and granulomatous tissues Bacterial contamination may change the clinical picture Peri anal infections and fistulas are a common disorder in adults and not uncommon in children Actinomycosis may be present in some of these cases The macroscopic examination may fail the bacteriological diagnosis often fails due to contamination with bacteria but the microscopic occurrence of ray fungus generally confirms the diagnosis

In the present case the site of invasion is unclear An invasion in connexion with superficial skin damage is a likely possibility A scratch on a lively child is generally un noticed No real trauma had occurred and moreover traumatic lesions of the perineal region are rare Abscess formation originating from small foreign bodies (pins tags fruit stones) enmeshed in the crypts of Morgagni may occur in infancy and childhood The actinomycetes organism is incapable of penetrating an intact mucous membrane However it becomes pathogenic when the barrier has been breached Thus the possibility of transrectal invasion cannot be excluded though proctoscopy at the operation was normal and no fistula was found

The prognosis in localized skin or cervicofacial actinomycosis is good When the lesions remain localized spontaneous cure may occasionally occur There have been numerous treatments recommended for actinomycosis i e sulphonamides penicillin X-ray therapy broad spectrum antibiotics etc Surgical evacuation of pus or necrotic material however is an essential part of the therapy Nevertheless if the condition is not diagnosed surgery like incision may open the possibility of lymphogenous or hematogenous invasion and thus a spread of the mycotic infection and a less favourable prognosis

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branches as it entered the liver. The cystic artery arose at the point of division of the right and middle hepatic. Within the parenchyma further dissection was abandoned.

No anatomical abnormality of the venous system was discovered, but a thrombus was present adherent to the wall of the umbilical vein passing on into the ductus venosus thus blocking the portal blood supply to the left lobe of the liver. A catheter in the umbilical vein passed through the ductus venosus and on into the inferior vena cava.

On microscopy the main branches of the hepatic artery were all patent. The portal tracts in the right lobe were smaller and fewer than in the left lobe. On cross section the hepatic arteries appeared smaller and also smaller in relation to the portal veins, 1 8 compared with 1 4 in the right lobe than the left (Figs. 2 and 3). The right lobe showed massive necrosis with some sparing of the parenchyma just under the capsule (Fig. 4) while the major part of the left lobe was intact apart from some small focal areas of necrosis see also Figs 2 and 3.

The lungs were firm and poorly aerated. Microscopy showed atelectatic areas and hyperaemia, in addition to widespread aspiration pneumonia. The other organs were normal apart from venous congestion and petechial haemorrhages in the pericardium.

DISCUSSION

In foetal life the physiological left lobe of the liver is supplied with oxygenated blood coming directly from the placenta, while the right side receives blood mainly from the portal veins (3). The lower oxygenation of the right lobe before birth makes it particularly susceptible to anoxic damage *in utero* (4) while the change over from oxygenated umbilical vein blood to portal blood at birth is said to be responsible for post natal involution of the left lobe. Due to these changes a line of demarcation may be seen between the physiological right and left lobes of the liver (3). The present case showed changes that were more severe than is usual with extensive necrosis of the right lobe and sparing of the left. These changes were associated with a difference in the calibre of the branches of the hepatic artery in the two lobes an arterio-portal ratio of 1 8 on the right compared with 1 4 on the left. The arterio-portal ratio in the adult is said to be constant at 1 8 while that in the newborn varies from 1 4 in the main brances to 1 1 in the smallest irrespective of lobe (6).

Infarction may follow the use of isotonic solutions in the newborn (11 9) but in the present case the thrombus in the umbilical vein and ductus venosus was probably associated with the presence of the catheter itself (1) as most of the solutions used were isotonic. Further as the thrombus blocked the portal blood supply to the left, but not the right lobe it cannot be held responsible for infarction of the latter. The differential diagnosis of massive hepatic necrosis (8) is unlikely in view of the normal liver weight and histological changes.

Zimmerman's case (13) of hypoplasia of the hepatic artery with hepatic infarction in an infant appears to be the only one on record. As in the present case there was ante mortem thrombus in the umbilical vein that continued into the ductus venosus blocking the opening of the portal vein and thus the supply of portal blood to the left lobe of the liver. The occurrence of infarction is attributed to the combined presence of arterial hypoplasia and lack of portal blood to the left lobe of the liver. A similar explanation, as is also involved in Kaplan's case (5) does not hold in the present case in which the arterial supply appears to have been the determining factor.

Fig. 1. Cut surface of the liver natural size showing the sharp line of demarcation between the necrotic physiological right lobe and the left lobe that is better preserved.

Fig. 2. Left lobe of the liver showing portal tract, size of hepatic artery (blood-filled) on cross-section related to portal vein, and intact hepatic parenchyma (Goldner's stain, 70).

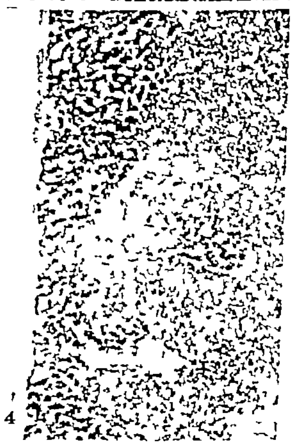
Fig. 3. Right lobe of liver showing portal tract, small size of hepatic artery (left) on cross-section related to portal vein, and necrosis of hepatic parenchyma (Goldner's stain, 70).

Fig. 4. Edge of necrotic area in liver showing preservation of parenchymal cells adjacent to liver capsule (Goldner's stain, ...).


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2


3


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Acta Pædiatr Scand 63 457–460 1974

CASE REPORT

CONGENITAL LACTOSE INTOLERANCE OF GASTROGEN ORIGIN ASSOCIATED WITH CATARACTS

GIUSEPPE RUSSO FLORINDO MOLLICA DOMENICO MAZZONE, and BENEDETTA SANTONOCITO

From the Paediatric Clinic University of Catania, Catania, Italy

ABSTRACT Russo, G., Mollica, F., Mazzone, D. and Santonocito, B. (Paediatric Clinic, University of Catania, Catania, Italy). Congenital lactose intolerance of gastrogen origin associated with cataracts. Acta Paediatr Scand, 63: 457 1974.—A case of congenital lactose intolerance with lactosuria associated with bilateral cataracts in a male infant is described. Lactosuria and cataracts were also present in four members of the two preceding generations.

According to per oral lactose tolerance tests evidence of transient intestinal lactase deficiency was found in the proband, and it was shown that lactosuria was related to the gastric passage of the lactose, since it disappeared when lactose was given intraduodenally

KEY WORDS: Lactose intolerance, lactosuria, cataracts

Severe congenital lactose intolerance first described in 1958 (7) is a rare disease which some authors (9 14) identify with congenital lactase deficiency while many others (1 5 10–12) consider it a separate entity The peculiar feature of severe lactose intolerance absent in congenital lactose deficiency is lactosuria though other distinctive features have been recognized signs of impaired renal function (hyperaminoaciduria, proteinuria, renal acidosis) (4 6, 7) a self-limited natural history with return to a normal lactose absorption after brief periods of lactose-free diet (4 11) and with normal lactase activity of the intestinal mucosa after "recovery" (1 10) and a rise in the blood glucose levels after oral lactose loads even in the active phase of the disease (6, 8 11)

The hypothesis of a congenital lactase deficiency of the intestinal mucosa does not explain satisfactorily any of these features. Recently it has been found (2) In an infant affected by severe congenital lactose intolerance that the disaccharidase activities of the small bowel including lactase were within the normal limits even in the acute phase of the disease and that lactosuria disappeared when lactose was administered intraduodenally through a feeding tube. A gastrogen origin of the disorder consisting of a abnormal resorption of the disaccharide(s) in the stomach has therefore been postulated (2)

We obtained similar results in a child we have recently studied In this child and in several of his relatives the lactose intolerance was associated with cataracts we do not know if this symptomatic association is incidental or if it represents a hitherto unrecognized syndrome

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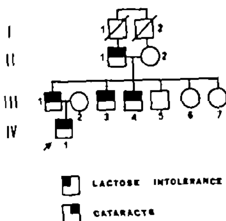


Fig. 2. The pedigree.

the father (III 1), two paternal uncles (III 3 and III-4), and the paternal grandfather (II-1). All these subjects were submitted to surgical treatment many years ago, with partial success only in II 1 the other subjects are blind from birth. Each of these subjects has excluded milk from his diet, because the ingestion of milk causes abdominal pains and diarrhoea The grandmother (II 2) remembers that she encountered some difficulties in feeding her first three children during their infancy and that she gave them "several types of milk, and also she-ass milk, because of digestive disorders

To each of the four adults with cataracts (II 1 III 1 III-3 and III-4), after overnight fast, 250 ml of homogenized cow's milk were given orally All complained of abdominal pains shortly after meal, and passed loose stools a few hours after In the urine collected in the following 24 hours a reducing sugar was present (from 0 5 to 1.2 g/l) that was identified as lactose by paper chromatography This test was also performed in a control group of eight unrelated healthy adults who were not milk drinkers, two of them had abdominal complaints a few minutes after the meal, but no appreciable lactosuria was present in the following 24 hours

DISCUSSION

The clinical and biological characteristics of our patient and particularly the lactosuria, are consistent with the diagnosis of familial lactose intolerance

The onset of diarrhoea in the first week of life and especially the presence of small but appreciable amounts of lactose in the stools both at admission and after reintroduction of the disaccharide to the diet, suggest an intestinal lactase deficiency But the rise in the blood glucose level after the second lactose load (see Fig. 1) clearly proves that the lactase deficiency was transient This agrees with the finding of normal lactase activity of the intestinal mucosa after the clinical recovery in patients affected by lactose intolerance (1 10) and makes hardly acceptable the hypothesis of an inherited or even a primary lactase deficiency

The apparently primary disturbance is bound to the passage of the lactose by the stomach Our experiments in agreement with the more prolonged observations of Berg et al (2) have shown that when lactose is given directly intraduodenally bypassing the stomach diarrhoea and lactosuria disappear

The suggested abnormal permeability of the gastric mucosa (2) could explain the lactosuria but the dependence of the diarrhoea from the gastric passage of the lactose remains unexplained An alternative hypothesis could be that in our patient an unknown factor related to the gastric passage interferes with the normal digestion of lactose by the intestinal mucosa this hypothesis would explain both the diarrhoea (osmotic) and the lactosuria (absorption of unsplitted lactose by intestinal mucosa)

If there is an abnormal gastric permeability it appears selective for lactose the flat glucose curve after the first oral load of galactose+glucose in our patient would denote that there is no anomalous permeability to glucose

Occurrence in siblings (6) and consanguinity of the parents (7) have been reported in cases of congenital lactose intolerance but the inheritance of the syndrome has not been yet definitely proved The family reported here is noteworthy in this respect. Four relatives of the proband from the two preceding generations complain of abdominal pains and diarrhoea, and present lactosuria, after ingestion of cow's milk Like that of the proband this disorder is congenital in appearance too since these subjects had digestive

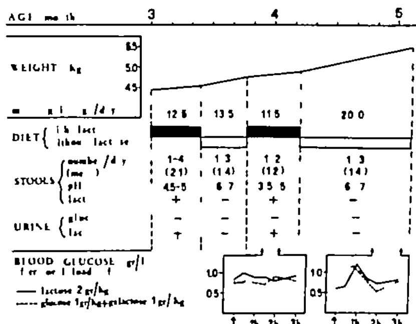


Fig 1 Congenital lactose intolerance. Relationship between the diet and some clinical and biochemical characteristics

nancy Delivery was complicated by premature rupture of the membranes. Birthweight was 3?00 g. Neonatal period was uneventful there was no jaundice The infant was breast fed in the first ? weeks after which pasteurized and partially skimmed cow s milk with added sucrose was given From the first week of life he suffered from moderate diarrhoea frequent vomiting and crying accompanied by tremors When he was 1 month old an EEG showed slight abnormalities, and an ophthalmologic examination revealed bilateral nu clear cataracts with horizontal nystagmus.

When 3 months old the infat weighed 4400 g and his height was 59 cm Apart from mild malnutrition and ocular signs the clinical examination did not reveal any pathological signs There was moderate diarrhoea with soft stools at low pH (4.5–5) Repeated EEG and ophthalmologic examination confirmed the previous findings

Appreciable amounts of reducing sugar (5 g/l) were found in the urine by Fehling test but Clinistix was negative The sugar was identified as lactose by unidimensional paper chromatography using a isopropanol water (4 1) solvent and aniline diphenylamine stain (13) Slight spots of sugars with Rf corresponding to lactose galactose and glucose were also present in the chromatogram of the stool. Fasting blood glucose levels ranged from 0.71 to 0 89 g/l in various determinations Moderate increase of BUN (0.55 g/l) was found Traces of protein were present in the urine Paper chromatography of the urinary aminoacids (15) after reference chromatography for creatinine revealed generalized hyperaminoaciduria without prevalence of single aminoacids. There was mild hypoproteinemia (5 15 g/100 ml) without dysproteinemia. Other routine laboratory tests gave normal results

During the following two months (see Fig. 1) the infant was given alternately powdered cow s milk with a normal lactose content (lactose intake about 4 g/kg/day) and a lactose-free diet (Galactomin formula 17) the caloric intake was constant during these ? months (about 130 cal/kg/day) The daily weight gain was higher and the number of stools lower during the lactose-free periods but the differences were slight. On the other hand the characteristics of the stools differed widely under the two regimens. they were much firmer and had higher pH (6–7 instead of 3.5–5) with the lactose-free diet Moreover the reintroduc tion of lactose in the diet resulted in the reappear ance of the lactosuria

During the third phase of this period (diet with lac tose) and during the fourth phase (lactose-free diet) blood glucose curves after oral loads of lactose (2 g/kg) and of galactose+glucose (1 g + 1 g/kg) were obtained. Both curves were flat in the third phase and both showed a good rise in the glucose levels in the fourth phase (see Fig 1)

The child spent the following 3 months at home he was given a diet without lactose was in good health and thrived regularly When 8 months old, he weighed 8080 g and his length was 70 cm. His stools were normal, with a pH superior to 5 5 there were no sugars in the urine The infant was then submitted to gastro-intestinal intubation end to end (3) and lactose (? g/kg) was administered intraduodenally under fluoroscopic control No lactosuria appeared in the following 24 hours nor when the procedure was repeated 3 days later But an identical amount of lactose administered twice orally was followed each time by lactosuria (lactose 0 5 and 0 6 g respectively in the urine discharged during the following 24 hours) and by diarrhoea.

The family

Fig. 2 shows the pedigree The paternal grandparents (II 1 and II 2) are first cousins. Besides the proband IV 1 four other members of the family are affected by bilateral cataracts and by horizontal nystagmus.

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CASE REPORT

COCKAYNE S SYNDROME IN TWO SISTERS

R. N SRIVASTAVA P C GUPTA, O MAYEKAR and S. ROY

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ABSTRACT Srivastava, R. N., Gupta, P C., Mayekar O and Roy S. (Departments of Paediatrics, Neurology and Pathology All India Institute of Medical Science New Delhi, India) Cockayne's Syndrome in tw sisters. Acta Paediatr Scand, 63: 461, 1974.—Clinical and laboratory data in two sisters with Cockayne's syndrome are presented. Both had severe dwarfism and mental retardation and the elder girl (11 years) had senile facies, optic atrophy and intracranial calcification. The serum cholesterol values were elevated. Peripheral neuropathy was found in both cases, evidenced by slow nerve conduction velocities. Examination of sural nerve biopsies showed focal demyelination with preservation of axis cylinders. These observations support the concept of Cockayne's syndrome being a leucodystrophy

KEY WORDS: Cockayne's syndrome, leucodystrophy peripheral neuropathy

Cockayne s syndrome is characterised by cachectic dwarfism severe mental retardation and a distinctive physical appearance consisting of senile facies loss of subcutaneous fat, a small head and disproportionately long extremities (1 6, 10) Other features include neurological abnormalities, ocular anomalies, deafness, dermal photosensitivity and skeletal changes (5) The syndrome has been established as a distinct entity and its homogeneity confirmed in about 30 published cases from different parts of the world (4 6 7 12) This report concerns observations in two sisters with Cockayne s syndrome

CASE REPORTS

Case I

M S an 11-year-old girl was referred for mental retardation and failure to thrive She was the product of a normal pregnancy and delivery and her early progress was satisfactory Failure to gain weight and unsteadiness of gait appeared at about the age of 2 years and abnormality of speech shortly afterwards. Her physical growth was extremely slow and at the age of 6 years it appeared to have ceased completely Increasing clumsiness made activities such as walking and feeding quite difficult. Her mental development remained slow A facial rash had been present intermittently Examination revealed a small, emaciated child with senile facies (Fig 1 and 2). The extremities were long the trunk short and the posture was one of generalised flexion (Fig. 1). Her weight was 9 4 kg (50th percentile for 1 year) and height 95 cm (50th percentile for 3 years). Other measurements were head circumference 43 cm, span 96 cm and vertex to pubes 45 cm. Scalp hair and nails were normal. Examination of abdomen, chest and cardiovascular system showed no abnormality

Neurological examination

Her speech was slow and slurred, in 3–4 worded sentences with marked dysarthria. The gait was spastic and she walked with support. Cranial nerves were intact and her vision hearing and response to painful stimuli seemed normal. There was moderate symmetrical wasting of the muscles of extremities accompanied by a marked increase in tone. Gross impairment of coordination was present.

disorders during their infancy and have from that time excluded milk from their diet

Systematic ophthalmologic examination has not shown ocular manifestations in the subjects affected by congenital lactose intolerance described up to now (8)

Five members of this family all with lactose intolerance present from their infancy bilateral cataracts and nystagmus the proband and four of his relatives Of course a fortuitous coincidence cannot be ruled out for the association cataracts–lactosuria but it is also possible that future systematic investigations in subjects affected by cataracts from infancy will reveal some abnormalities of the lactose absorption and/or metabolism

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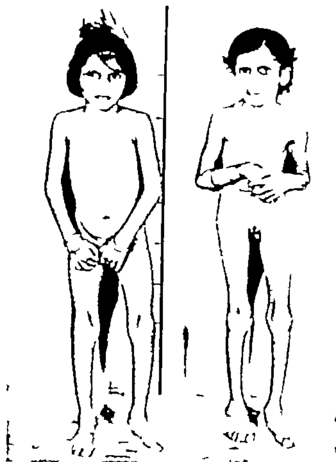


Fig 1 Case II (41/2 years) and Case I (11 years) showing short trunk long extremities and an attitude of generalised flexion

There were no abnormal movements Tendon reflexes were brisk and the plantars equivocal Optic fundi showed primary optic atrophy Her I Q was estimated to be 20

Case II

E S younger sister of M S was first seen at the age of 41/2 years She had developed normally for 2 years when abnormalities of gait and speech appeared and gradually worsened Subsequent physical as well as mental progress was extremely slow She looked small for her age. The facial appearance was relatively normal (Fig 1) Her weight was 10 8 kg and height 93 cm (50th per centiles for 16 and 33 months respectively) head circumference 42 cm span 93 cm and vertex to pubis 47 cm. Examination of the abdomen, chest and heart was unremarkable Scalp hair skin teeth and nails were normal Neurological examination revealed abnormalities of gait, speech and motor system essentially similar to those of the elder sister but of a milder nature Her hearing vision and optic fundi were normal. The I Q was 44

Family and follow-up

The father (42 years) and the mother (37 years) were healthy and unrelated. Their three sons aged 13 10 and 7 years and a daughter of 8 months were normal. Both patients were observed for 31/2 years during which progression of neurological abnormalities was seen. The elder girl had become bed-ridden with contractures and developed impairment of hearing The progeroid appearance was becoming manifest in the younger girl

Laboratory studies

The results of the following investigations in both patients showed no abnormality: haemogram, serum electrolytes, calcium phosphorus alkaline phosphatase SGOT SGPT total and differential proteins creatinine immunoglobulins and complement fasting blood sugar protein-bound iodine 24-hour urinary ketosteroids and ketogenic steroids urinary aminoacid chromatogram. Urinary sediment did not show metachromatic granules Serum cholesterol values were elevated being 400 mg/100 ml and 310 mg/100 ml in the patients I and II respectively Examination of cerebrospinal fluid (protein 30 mg/100 ml) skin biopsy and chromosomal pattern in case I showed no abnormality Radiological examination showed intracranial calcification in occipital region in the first case Bone age corresponded with chronologic age in both cases. A pneumo-encephalogram was done in the first case and revealed cortical thinning and symmetrical ventricular dilatation. Electroencephalogram was abnormal in both cases showing excess of slow activity which was more prominent in case I with an



Fig 2 Facial appearance of Case I showing senile features

Table 1 *Peripheral nerve conduction studies*

Normal values. Motor conduction velocities (ulnar and median nerves): 56 1±4 7 metres per second. Terminal latency 3 8±0 5 millisecond. Sensory action potential: 2.5 to 4 millisecond

Patient	Nerve	Motor conduction velocity: Axilla to elbow (metres/sec)	Elbow to wrist (metres/sec)	Terminal latency (millisec)	Sensory action potential. Wrist to finger (millisec)
I	Ulnar	30	–	–	–
	Median	29	20	3.2	4.4 Low voltage poorly formed
II	Ulnar	19	41	3.0	–
	Median	21	40	3 9	5 Well-formed

occipital rhythm of 5–6 cycles per second than in case II with 7 cycles per second.

Nerve conduction velocities

These were determined in the left ulnar and median nerves. Marked slowing was observed in both cases (Table 1) Sensory action potentials were delayed and poorly formed Prolonged and repetitive discharges were obtained following stimulation of the nerve. H-reflex was elicited in ulnar nerve in case I.

Nerve biopsy studies

Examination of sural nerve biopsies from both patients revealed changes that were similar but more severe in case I. Light microscopic evaluation showed foci of swelling of myelin sheath and of complete demyelination (Fig 3). There was a mild increase in perineural connective tissue. A few Schwann cells contained P A S.-positive granules which did not give metachromatic reaction to toluidine blue stain. Axis cylinders did not show significant change Electronmicroscopy confirmed severe degenerative changes of myelin sheath without significant abnormality of the axons (15)



Fig 3 Sural nerve biopsy from Case I showing degeneration of myelin sheath in several nerve fibres (Weil-Weigert stain, x310).

DISCUSSION

Clinical features of Cockayne s syndrome are quite distinctive and a comparison of the photographs of the patients reveals a striking similarity (6, 8) The patients are usually normal at birth and remain so till late infancy when symptoms appear Evolution and progression of some of the features was noted in the present cases Gross impairment of vision and hearing was initially absent in both and the senile facies in the younger girl These developed during subsequent observation. Laboratory studies, except for raised values of serum cholesterol were unremarkable in these patients A wider involvement in this syndrome is suggested in view of the reported abnormalities of renal function (5) and renal histology (12 14) hyperlipoproteinemia (5) and defective glucose metabolism (2 5) in addition to the well-known changes of skeleton and skin (6 13) Familial occurrence of Cockayne s syndrome (1 6 10 13) and parental consanguinity (13) have been observed and an autosomal recessive mode of inheritance suggested Sporadic examples have been also recorded (3 8 12) No chromosomal abnormality has been found (8 13)

Pathology of the brain in Cockayne s syndrome has been examined by several workers (3 9 11 14 16) Microcephaly cortical thinning, shrinkage of brain stem and cerebellum ventricular dilatation and calcification in the basal ganglia are prominent fea-

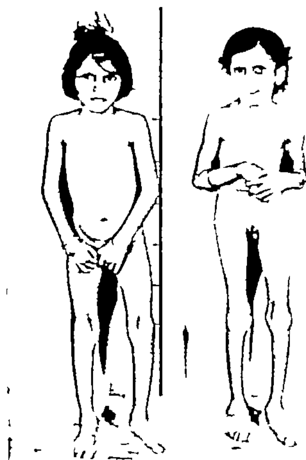


Fig 1 Case II (41/2 years) and Case I (11 years) showing short trunk long extremities and an attitude of generalised flexion

There were no abnormal movements Tendon reflexes were brisk and the plantars equivocal. Optic fundi showed primary optic atrophy Her I Q was estimated to be 20

Case II

E S younger sister of M S was first seen at the age of 41/2 years She had developed normally for 2 years when abnormalities of gait and speech appeared and gradually worsened. Subsequent physical as well as mental progress was extremely slow She looked small for her age The facial appearance was relatively normal (Fig. 1) Her weight was 10 8 kg and height 93 cm (50th per centiles for 16 and 33 months respectively) head circumference 42 cm span 93 cm and vertex to pubis 47 cm. Examination of the abdomen, chest and heart was unremarkable. Scalp hair skin teeth and nails were normal Neurological examination revealed abnormalities of gait, speech and motor system essentially similar to those of the elder sister but of a milder nature Her hearing vision and optic fundi were normal. The I Q was 44

Family and follow-up

The father (42 years) and the mother (37 years) were healthy and unrelated. Their three sons aged 13 10 and 7 years and a daughter of 8 months were normal Both patients were observed for 31/2 years during which progression of neurological abnormalities was seen. The elder girl had become bed ridden with contractures and developed impairment of hearing. The progeroid appearance was becoming manifest in the younger girl

Laboratory studies

The results of the following investigations in both patients showed no abnormality: haemogram serum electrolytes, calcium phosphorus alkaline phosphatase SGOT SGPT total and differential proteins creatinine immunoglobulins and complement fasting blood sugar protein-bound iodine 24-hour urinary ketosteroids and ketogenic steroids urinary aminoacid chromatogram. Urinary sediment did not show metachromatic granules. Serum cholesterol values were elevated, being 400 mg/100 ml and 310 mg/100 ml in the patients I and II respectively Examination of cerebrospinal fluid (protein 30 mg/100 ml) skin biopsy and chromosomal pattern in case I showed no abnormality Radiological examination showed intracranial calcification in occipital region in the first case Bone age corresponded with chronologic age in both cases A pneumo-encephalogram was done in the first case and revealed cortical thinning and symmetrical ventricular dilatation. Electroencephalogram was abnormal in both cases showing excess of slow activity which was more prominent in case I with an



Fig 2 Facial appearance of Case I showing senile features

Acta Pædiatr Scand 63 465–471 1974

CASE REPORT

HEREDITARY RECURRENT CHOLESTASIS WITH LYMPHOEDEMA—TWO NEW FAMILIES

Ø AAGENÆS

From the Paediatric Department University Hospital Oslo Norway

ABSTRACT Aagenaes, Ø. (Paediatric Department, University Hospital, Oslo, Norway). Hereditary recurrent cholestasis with lymphoedema—two new families. Acta Paediatr Scand, 63: 465, 1974.—Hereditary neonatal cholestasis with primary lymphoedema at birth or later have earlier been described in two families of Norwegian origin. This report includes the clinical course and the liver histology in two new cases with this syndrome. The two patients were unrelated and unrelated to the earlier described families. Both had neonatal intrahepatic cholestasis, in one lasting many years, in the other only about 6 months. Lymphoedema was severe in the first mentioned and more moderate in the latter Liver histology showed giant cell transformation in infancy and some fibrosis or cirrhosis in later childhood. In the first family 3 of 6 siblings were similarly affected at birth, and in the other family the parents were related, supporting earlier theory of an autosomal, recessive heredity as the aetiology of this condition.

KEY WORDS: Neonatal cholestasis, lymphoedema

Since our reports in 1968 and 1970 (1 2 8) where we described a large pedigree with recurrent cholestasis beginning in infancy and lymphoedema in the lower extremities beginning in later childhood only one report has appeared with the combination of cholestasis and lymphoedema (7) We have been following the development of two other patients with this syndrome through childhood in recent years and because of the paucity of reports on this syndrome it seems worthwhile to add these new families to those already described

Case 1

R. S., boy born September 1962. The father of the patient is first cousin of his own mother-in-law (Fig. 1) B W 3510 g. B.L. 51 cm. Jaundice first week, increasing from 4 weeks of age, light-colored stools, dark-colored urine. Admitted Paediatric Department University Hospital 3½ months of age with suspicion of biliary atresia. Liver palpable 4 cm below costal margin. No splenomegaly Stools not completely acholic. Explorative laparotomy showed normal biliary tree, but sticky bile in gallbladder and biliary tree. After rinsing with saline contrast could be injected from gallbladder to duodenum. Obstructive jaundice continued the following years, and two years after the first laparotomy the boy had a second exploratory operation. The liver was firm, reddish brown Normal biliary tree.—Serum albumin was low prior to the operation (2.7 g/100 ml) and de

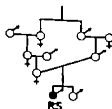


Fig 1 Pedigree of Case 1 R. S

tures Microscopically there is extensive peri vascular calcification being heaviest in the basal ganglia Patchy demyelination in a markedly reduced white matter is a striking feature Axis cylinders are preserved and there is mild gliosis

Moosa & Dubowitz recently reported peripheral neuropathy in a patient with Cockayne s syndrome (8) They remarked upon the clinical similarities between this syndrome and various leucodystrophies and considering patchy demyelination as the pre-eminent neuropathologic feature supported the earlier postulation that Cockayne s syndrome could be a form of leucodystrophy (11) Seitelberger (16) has regared this syndrome as a special variant of symptomatic Pelizaeus Merzbacher disease (an orthochromatic leucodystrophy) in view of the morphologic similarity of the demyelination pattern in these two disorders Presence of peripheral neuropathy and demyelination of peripheral nerve in the present cases would favour the concept of Cockayne s syndrome being a leucodystrophy

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CASE REPORT

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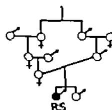


Fig 1 Pedigree of Case 1 R. S.

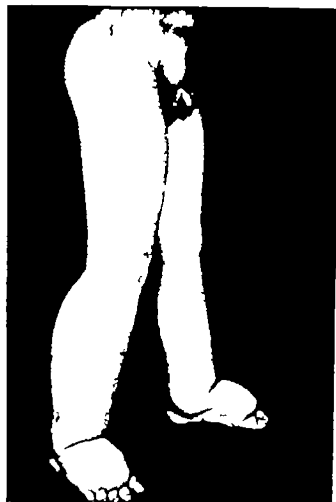


Fig 2 Severe lymphoedema in both lower extremities in Case 1 R. S

creased further (? 2 g/100 ml) and he had a pronounced *oedema which disappeared under treatment* with albumin and diuretics. Readmitted at age 4½ years —still moderately jaundiced Now permanent oedema on lower extremities. Jaundice disappeared between 7 and 9 years of age Improving condition no steatorrhoe at the last admission age 10 years but still permanent lymphoedema in the lower extremities (Fig. 2).

Lymph-vessel screening with subcutaneous injection of dye on the back of the foot showed pronounced dermal back flow On two occasions highly experienced investigators were unsuccessful in their efforts to find a lymphvessel for a lymphangiography Laboratory data are shown in Table 1 Until age 10 there were signs of cholestasis with hyperbilirubinemia, hyperlipemia, high alk phosphatases small changes in serum proteins and steatorrhoea Clinically the patient complained of moderate pruritus. At age 10 all data were nearly normalized.

Fig. 3 shows the growth curve of the child. There was a growth retardation in the first two years of life. Since that time the growth velocity has been in the normal range for age but without any good catch-up-growth.

Liver biopsy at age 3½ months of age showed a pronounced giant cell transformation moderate cellular infiltration some necrosis and bile retention in parenchymal cells and bile canaliculi (Fig 4).

Repeated liver biopsies at age ? and 7 years showed decreasing giant cell transformation and cholestatic signs a needle biopsy at age 10 years showed increased fibrosis and probably rebuild architecture (Fig 5).

Case 2

S E girl born April 1955 (Pedigree Fig. 6.) She is number 5 of 6 The two oldest siblings, both boys, born in 1938 and 1940 had jaundice from birth light-colored stools and dark urine No medical examination died at home at age 6 weeks. No bleeding tendency observed. B W 3500 g. B L. 51 cm Jaundice from few days of age, light-colored stools, dark urine Admitted Paediatric Department University Hospital at 2 months of age. Liver enlarged 4 cm below costal margin no splenomegaly During a 3-month stay in our department, where laparotomy was considered jaundice decreased slowly and no laparotomy was performed In the following years only slight hyperbilirubinemia, but com*plained of pruritus now and then At five years of age* increasing pruritus, followed by jaundice This icteric period lasted for 2-3 months At the end of this period a laparotomy was performed, and a normal biliary tree was found In spite of this an anastomosis between the gallbladder and duodenum was performed.

From age 11 tendency to swollen ankles (Fig 7). At

Table 1 *Laboratory data on patient R S*

Age	4 months	15 months	28 months	5 years	7 years	10 years
Hemoglobin g/100 ml	10 3	12 0	12.0	11 0	11 8	10 8
Bilirubin mg/100 ml	4 9	4	12	3 1	4 2	1 6
SGOT K U	100–140	75–100	100	75–50	25–100	20
Alk phosph	Increased	Increased	Increased	Increased	Increased	Slightly increased
Albumin g/100 ml	3 0–4 2	3 7	2 7–2 2	3.8	3 6	3 4
α2-glob g/100 ml	0.6–0.8	0 9	1 0–1 4	1 1	1 1	0 6
β-glob g/100 ml	0.9	0 9	1 1–1 2	0.8	1 2	0.8
Cholesterol mg/100 ml	226		403	218–302	347–?45	155
Triglycerids mg/100 ml				38–132	180	
Phospholip mg/100 ml				193–280	446–230	157
Prothrombin %	100		100	90	85	80
Stool-fat, g/day	11 4			14 7	8 2	4 9

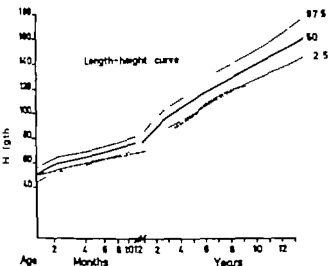


Fig. 3. Growth curve of Case 1. R. S. The 2.5, 50 and 97.5 percentile lines are also outlined.

age 13 a new period with jaundice and pruritus, light stools and dark urine, duration 2–3 months. Similar periods with obstructive jaundice at age 16 and 17.

The tendency to oedema in the legs has increased and the circumference of the legs was about 43 cm at age 16½. After treatment with diuretics, elastic bandage etc. the circumference decreased 2–3 cm, and the weight decreased 8 kg. No relationship between periods of jaundice and increase in oedema. A lymphangiography on the one leg showed fewer lymphvessels than normal but no pronounced lymph pathology.

Laboratory data are shown in Table 4. It is seen here

Fig. 4. Liver biopsy from R. S. at age 3½ months. Nearly complete giant cell transformation, many of which are in a lytic state.

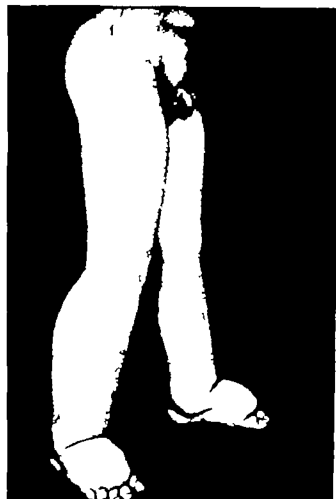


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Triglycerids mg/100 ml				38–132	180	
Phospholip mg/100 ml				193–280	446–230	157
Prothrombin %	100		100	90	85	80
Stool-fat, g/day	11 4			14 7	8 2	4.9

Table 2. *Laboratory data on patient S E.*

Age	2 months	7 months	15 months	5 years	6 years	13 years	14 years
Hemoglobin, g/100 ml	12	1	11	11.6	11	1..6	1..6
Bilirubin, mg/100 ml	11	1.5	0.8	10 6	0 1	11 9	0..
SGOT K.U				120	35	31	20
Alk. phosph.				Increased	Normal	Increased	Normal
Albumin, g/100 ml	4 4	4 4	4 6	2.5	3 1	4 0–3 0	3.3
α_2-glob g/100 ml				0.8	1 1	0 7–0 9	0.7
β-glob., g/100 ml				1 4	1 1	0.9–1..	0.7
Cholesterol, mg/100 ml			140			350	222
Triglycerids, mg/100 ml						239	57
Phospholip. mg/100 ml						280	167
Prothrombin %	55		90	90	100	80	77
Stool-fat, g/day			4.6				

in the two patients (Tables 1 and 2) Hyperbilirubinemia, hyperlipemia (both cholesterol, triglycerids and phospholipids—and lipoprotein electrophoresis showed mainly increased β-lipoproteins) increased transaminases and alk. phosphatases in the cholestatic phases and nearly normal values outside the cholestatic phases

Serum proteins show small changes During periods with severe cholestasis serum albumin tends to be low and α_2- and β-globulins tend to be moderately increased. During anicteric periods serum proteins are mainly in the normal range

In periods with cholestasis increased content of fat was found in the stools and in anicteric periods the amount of fat in the stools was close to normal.

All these laboratory changes can well be explained by the cholestasis per se

Tendency to oedema started as early as 3–4 years of age in R. S and he has developed a severe lymphoedema later on (Fig. 2) The studies of the lymph vessels in the lower extremities point to a severe lymph vessel hypoplasia.

In patient S E. the tendency to oedema in the lower extremities was first recognized at age 11 and the oedema can be kept moderate with treatment (Fig. 7) Lymphangiography showed only moderately reduced lymph vessels in the one studied extremity Heart examination and blood pressure were normal in both patients.

The histology of the liver biopsies was dominated by giant cell transformation mainly in infancy (where a biopsy was performed at this age) and cholestasis. Later on a

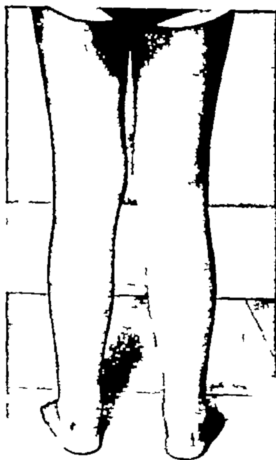


Fig 7 Moderate lymphoedema in Case 2, S E.

Fig 5 Liver biopsy from R S at age 10 years, showing increased fibrosis and probably rebuild architecture—cirrhosis?

that the cholestatic phases have been relatively short, with nearly normalization of liver values in between the cholestatic phases

The growth of this child has been in the normal range

Liver biopsy at age 6 years showed some giant cell transformation and slight increase in connective tissue

A new biopsy at age 13 years in a cholestatic phase showed bile pigment in Kupffer cells, in hepatocytes and in bile canaliculi and still only slight fibrosis

DISCUSSION

In our first publication (1) we concluded that an autosomal recessive inheritance was most probable in the reported family These two patients appear to conform rather well with this conclusion There is close consanguinity between the parents of R S and in the second family 3 of 6 siblings seem affected

We have very little data on the two older brothers of patient S E but we can conclude that they had a cholestatic picture in infancy The reason for their deaths is unknown but based on data from our other large family (1) internal bleeding may well have been the cause These boys were not treated with vitamin K (born 1938 and 1940)

The duration of the intrahepatic cholestasis was much longer in patient R S than in patient S E The growth curves of the two patients reflect this fact patient R S being severely growth retarded and patient S E growing in the normal range

The laboratory data are principally similar

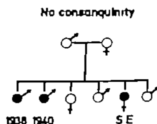


Fig 6 Pedigree of Case 2 S E.

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more or less pronounced fibrosis and in case R S a severe fibrosis was found The devel opment of fibrosis seems slow—and no signs of portal hypertension have been found

The laboratory data the liver histology and the lymphoedema in these two patients indicate that these patients have the same syndrome that we have described earlier (1 2 8) As far as we can see they are not related to each other or to the previously de scribed large family

The only other patients described with the combination of cholestasis and lymphoedema are those published by Sharp et al (7) They had lymphoedema from birth and the liver disease seemed somewhat more severe than the liver disease in our patients

We still have no good explanation for the relationship between the lymph pathology and the cholestasis in these patients Thor ough studies by light and electronmicroscopy both by Sharp et al (7) and by our group (unpublished data) have not revealed any lymph vessel pathology or sinusoid pathology in the livers of these patients but as both quantitative and qualitative studies of the histology of the lymph vessels in the normal livers are virtually impossible based on avail able technical methods any pathology has to be very pronounced to be recognized — We have previously found (8) that the lymph drainage from the subcapsular space of the liver was pathological in one of our adult patients in a period without cholestasis but as the patient had some liver fibrosis it is impossible to say whether this pathological lymph drainage is primary or secondary to the fibrosis

In a recent work (3) we have studied the effect on bile flow by an artificial liver lymph obstruction in cats We found an increase in the bile flow of relatively short duration Ear lier studies (6) have shown that obstruction of the biliary tree leads to an increase in the lymph flow from the liver These studies show that close relationship between the bile ductuli and the lymph vessels (4) is not only an anatomical relationship but a functional one as well

We are therefore of the opinion that lymphhypoplasia (undetected because of tech nical problems) or a functional lymph defect in the liver might explain the cholestasis in these patients

Earlier studies on early and late primary lymphoedema (5) have suggested that the pathogenesis in both types of primary lymph oedema is a congenital lymph vessel patho logy but of different type and location. If our suggestion on the pathogenesis of the cholestasis is correct there should be no dif ficulty in explaining why some patients have early lymphoedema (as in Sharps family (7)) and others have late lymphoedema

In a recent work Watson & Miller (9) have described an hereditary disease from Eng land where the patient had a combination of neonatal cholestatic liver disease and vascular malformations mainly as hypoplasia of the pulmonary arteries An autosomal dominant inheritance with variable penetrance was sug gested

The British authors consider a primary de fect in the hepatocyte-canalicular region as the cause of the hepatic manifestations A similar cause in the Norwegian type could not explain the pronounced degree of recur rent cholestasis in our patients which is why we have to suspect a more functional abnor mality

Our families and Sharp s family are all of pure Norwegian descent In view of the in creasing number of reports on hereditary cholestasis being published in recent years we suggest the name hereditary cholestasis of Norwegian type for those combined with lymphoedema.

In an earlier publication (1) we suggested a good prognosis for patients with this con dition In the meantime one of our earlier reported cases has died of liver failure outside the hospital and the liver fibrosis seems se vere in case 1 R S The prognosis is there fore not always favorable

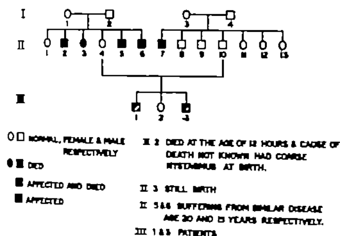


Fig 2 Family tree showing sex-linked transmission

He could creep at the age of 2 1/2 years and started speaking by 3 years. The child started to walk unaided at the age of 3 1/2 years and is able to run for short distances at present. The younger child learned to sit at the age of 16 months but cannot creep stand or walk The sister aged 3 years is physically and mentally normal

Two maternal uncles aged 20 and 30 years were found to have oscillating movements of the eyes They were reported to have been able to sit after 2 years of age but cannot stand without support The disease remained static and no progress in neurological deterioration was observed over the years, either in the two brothers or in their uncles. The family tree is shown in Fig 2. No consanguinity was recorded.

Physical examination of both patients showed coarse nystagmus, intention tremor truncal ataxia and signs of in-coordination in the upper extremities. Signs of pyramidal tract involvement were present in the lower limbs. Cranial nerves including fundi were normal Both had a moderate degree of mental retardation (I Q 60). Examination of maternal uncles showed similar features and they were also mentally retarded

INVESTIGATIONS

The investigations performed in the two brothers are given in Table 1

DISCUSSION

Ataxic syndromes in cerebral palsy include congenital ataxia and ataxic diplegia The ataxic diplegia constitutes about 5–7% of all cases of cerebral palsy (2) Fried (4) first distinguished the ataxic form of cerebral palsy from other varieties and Lund (6) noticed that in some cases of congenital cere bellar ataxia there might be features of spas ticity in addition to the cerebellar signs Subsequently Ingram (3) termed cases with cerebellar ataxia and spastic pareses in

Table 1 *Laboratory findings in two patients with familial ataxic diplegia*

	D M	K. M
Haemoglobin, g/100 ml	10.5	11.5
Leucocyte count/mm³	14 000	10 000
Differential leucocyte count	Polymorph 55% Lymphocyte 35% Eosinophil 10%	Polymorph 45% Lymphocyte 55%
Leucocyte morphology	No abnormality observed	No abnormality observed
Urinary amino acids	Normal pattern	Normal pattern
Cetyltrimethyl ammonium bromide test for excessive urinary mucopolysaccharides	Negative	Negative
X-ray of skull	Normal	Normal
β-lipoprotein, mg/100 ml	125	150

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CASE REPORT

FAMILIAL ATAXIC DIPLEGIA

G SUBRAHMANYAM A M TRIPATHI and K N AGARWAL

From the Department of Paediatrics
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Varanasi India

ABSTRACT Subrahmanyam G., Tripathi, A M and Agarwal K. N (Department of Paediatrics, Institute of Medical Sciences, Banaras Hindu University Varanasi, India) Familial ataxic diplegia. Acta Paediatr Scand, 63 472, 1974 —A family with ataxic diplegia, mental and physical growth retardation in two generations is presented. The disease was limited to male members, suggesting a sex-linked transmission.

KEY WORDS Familial ataxic diplegia mode of inheritance

Ingram (3) defined ataxic diplegia as cerebellar ataxia associated with spastic pareses mainly of lower extremities Bille & Hagberg (1) reported ataxic diplegia in a girl of 16 years and her brother of 12 years It was associated with severe form of mental defi ciency The parents were normal Gustavson et al (2) reported three families with ataxic diplegia in one family three subsequent ge nerations were affected The paucity of clini cal reports stimulated us to report a family of ataxic diplegia in two generations

CASE REPORTS

Two brothers D M 5 1/2 years and K M 1 1/2 years of age (Fig. 1) were admitted with the complaints of unsteadiness delayed milestones and nystagmus Both were born after normal pregnancy and delivery The birth weights were not recorded. They had oscillating movements of both eyes since birth. When grasping for objects tremor appeared which was absent at rest Neck control in both children was achieved around 10 months of age The elder started recognising the mother at the age of 15 months learned to sit and to stand with support after physical training at the age of 2 years



Fig 1 Brothers KM 1 1/2 and DM 5 1/2 years

PROCEEDINGS OF PAEDIATRIC SOCIETIES

SWEDISH PAEDIATRIC ASSOCIATION

MEETING IN LINKÖPING MARCH 24 1973

Y Larsson & J Ludvigsson *Perinatal mortality in diabetic pregnancy*

An analysis has been carried out of 191 diabetic pregnancies observed over a period of 5 years at four hospitals in the region of Linköping. Legal abortion was done in 16 cases spontaneous abortions occurred in 19 cases Among the remaining 157 cases 35 children died perinatally giving a perinatal mortality of 22.3% The perinatal mortality was not significantly correlated to the degree of severity of the mother s diabetes but was correlated to the blood sugar level during the last weeks of pregnancy Among live-born children hyperbilirubinemia occurred in 29.5% IRDS in 9 8% hypoglycemia in 47.5% and macrosomia in 43 4% The importance of an intensified diabetic control during pregnancy was emphasized and the organizational measures necessary for this purpose were discussed

S Bremberg: *Adolescent mothers in Linköping*

A high frequency of complications, both medical and social has been reported from the Uk and US for girls who bear children in their teens. The present study was undertaken to find out whether similar problems occur in Sweden

Some 624 subsequent deliveries in 1968–1971 of primiparae aged 19 or below were studied and the outcome of these deliveries was compared with those of primiparae aged 20 or above A delivery without any complications was more common in young mothers particularly in those aged 17 or below The perinatal mortality did not differ between the groups Yet the frequency of preterm deliveries was two times higher for mothers aged 17 or below

Questionnaires regarding socio-economic conditions were sent to 104 teenage mothers with children aged 1–2 years and to control mothers aged 21–29 years Both groups had similar housing standards. Most teenage girls were living with their husbands/fiancés (82%) The median income of these families amounted to half the income of the older families Welfare subsidies affected this difference slightly About 10% had left school due to their pregnancy Three times as many of the mothers in their 20 s had completed college education compared with the teenage mothers but the number who had completed vocational school was the same in both groups

The medical problems for these teenage mothers seem to be limited. Yet these families have considerable economic difficulties

A. Häger *Insulin secretion and cellularity of adipose tissue in obese schoolgirls*

Obesity may be defined as an increase in the total number of cells (hyperplasia) and/or an

lower limbs as Ataxic diplegia Familial occurrence has been reported (1 2) with male and female members equally affected A sexlinked transmission has hitherto been described only by Wolfslast (7) The family presented in this report also shows a sex linked transmission of the disease with affected males in two generations

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India

first time, it was also possible to demonstrate wide pulp chambers in the permanent teeth

Hypophosphatasia must be suspected in cases of severe skeletal deformation and rickets in children with convulsions and in all cases of craniosynostosis Of particular importance is the possibility of hypophosphatasia in children with premature loss of primary teeth

There is no causal treatment but the patients must be protected from overdosage of vitamin D Genetic studies should be made and the social problems of the families have to be considered.

Vera Oldfelt. *School performances of children with low birth weight*

During the period 1958–61 6330 children were born in Linköping and out of these 320 had a birth weight of 2500 g or less 69 children died during the neonatal period and the first year and 7 at a later date Death rate was 24% 57 children had a birth weight of 1500 g or less and of these 45 died Mortality was 80%

Some 244 children were monitored until at least 11 years of age and their school performances evaluated

The series was divided into three groups according to the severity of the perinatal situation. In the most vulnerable groups as many as 80% of the children had normal school performances

Severe and moderate handicaps (according to M Drillien) were observed in 8.5%

There was no statistical difference in the school performances between children classified at birth as pre-term small for date or dysmature babies

Anna-Greta Malmström-Groth *Hereditary Cerebral Palsy—a theme with variations*

A brief account is given of the literature concerning the syndromes of cerebral palsy Approximately 1.5% of CP-syndromes in Sweden are calculated to be autosomally recessively inherited The sex linked recessive or dominant forms are much less common

Six affected children from 3 families are reported

1 Two siblings a girl and a boy both premature with spastic diplegia, normal intellectual capacity and no proven immunological defect (DNCB-test not done)

2. Another pair of siblings a boy and a girl with cerebellar hypoplasia confirmed by X ray slight mental subnormality and no chromosomal defect Symptomatologically they belonged to the intermediate group between congenital cerebellar ataxia and the dysequilibrium syndrome

3 A 7 year-old girl who had initially been thought to be suffering from static CP with ataxia and mental retardation At the age of 4–5 years she developed signs of progressive encephalopathy of unknown origin What is going to happen to her 4-year-old female second cousin with the diagnosis of static congenital slight ataxia and mental retardation?

G Sanner *The genetics of cerebral palsy*

On the basis of literature reports and own investigations the significance of genetic etiology and the proportion and probable mode of inheritance in the different syndromes of cerebral palsy were discussed

Yvonne Ringheim Anna-Greta Malmström-Groth Franjo Farago *Infantile spasms with hypsarrhythmia The role of hereditary factors*

Abnormal electroencephalograms have been encountered in family members of patients with petit mal epilepsy and in patients with psychomotor epilepsy

In the present investigation the electroencephalograms of members of the families of 11 children with infantile spasms were col

enlargement of the adipocytes (hypertrophy) If untreated 80% of obese children remain fat into adult life and make up 50% of adults with severe overweight Hence overweight in childhood remains a challenge to the pedia trician

Preliminary data from a study of 30 grossly obese girls and 30 control cases were pre sented The method used included

1 anthropometric estimations (skin fold measurements etc)

2 estimation of total body fat in a whole body counter (^{40}K)

3 percutaneous needle biopsies for adipose cell size measurements

4 early insulin response during an i v GTT

5 simulated early insulin response test (according to Thorell) This test measures the decrease in blood glucose after induced transient hyperinsulinaemia at physiologic levels in the posthepatic circulation

The results demonstrate that the adipose tissue of obese girls (7–10 years of age) exhibits both an increase in total cell number and an enlargement in cell size sometimes above normal adult levels High insulin levels were demonstrated in the i v GTT With the simulated early insulin response test it could be shown that the hyperfunction of the beta cells was due to a peripheral insulin resis tance The tests and measurements will be repeated after weight reduction

N I M Kjellman & Y Larsson *Insulin release in cystic fibrosis*

Early insulin response (ER) to rapid intravenous injection of glucose was studied in 7 cases of cystic fibrosis aged 0 8–9 5 years Plasma insulin was measured with a radioimmunological method Blood glucose values were determined and K_G-values calculated

In all children except the youngest the ER values were very low as compared with nor mal children and the K_G-values were mod erately decreased ER and K_G-values were not correlated to the duration of the clinical symptoms nor to the patients actual clinical condition as measured by means of the Shwachman score

The explanation of the decreased insulin response and the increased incidence of diabetes in cystic fibrosis is probably the progressive fibrosis of the pancreas Comparison was made with the conditions in pancreatic fibrosis in rabbits produced through duct ligation

N I M Kjellman Vera Oldfelt Å Nordenram & L. Rabow *Hypophosphatasia*

Hypophosphatasia named by Rathbun in 1948 is an inborn error of metabolism with deficiency of alkaline phosphatases in blood and tissues About 140 cases have been published hitherto An increased urinary excretion of pyrophosphate and phosphoethanol amine can usually be demonstrated Rickets like symptoms and premature loss of primary teeth are the main clinical manifestations of the milder forms of hypophosphatasia. About a third of the cases are more malignant with convulsions and renal failure especially after therapy with vitamin D

During the last decade we have diagnosed 5 cases of hypophosphatasia Two of the children were siblings Their symptoms began at a mean age of 0 6 years and diagnosis was made at a mean age of 2 3 years

Craniosynostosis requiring surgical treat ment was revealed in 2 cases and should be sought for in every case of hypophosphatasia.

Convulsions were present in 2 cases Irritability and skeletal pain predominated during the first years as well as the rickets-like changes With increasing age all the symptoms abated as demonstrated by our oldest patient where the only findings now are flatfeet and a slight lumbar lordosis

All the children had premature loss of pri mary teeth Typical dental findings with wid ening of the pulp chambers and aplasia of root cementum were demonstrated For the

rst time, it was also possible to demonstrate ide pulp chambers in the permanent teeth

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Abnormal electroencephalograms have been encountered in family members of patients with petit mal epilepsy and in patients with psychomotor epilepsy

In the present investigation the electro- encephalograms of members of the families of 11 children with infantile spasms were col-

lected The records of 7 of 26 parents and siblings showed generalized paroxysmal activity

In 5 of 11 families at least one family member (apart from the patient) had an abnormal electroencephalogram or manifest epilepsy

A genetic factor as demonstrated by electroencephalograms may be an etiological factor in some cases of infantile spasms

MEETING IN LUND MAY 1? 1973

G Blennow J Lagergren & Margareta Jägerstad *Is there any deficiency in folate and B_{12} in fenytoin treated Swedish children?*

There are several hazards in long-term anti epileptic drug treatment At the moment a study is in progress to ascertain the frequencies of folate deficiency disturbed calcium metabolism disturbed immunglobulin concentration disturbed liver function and also the correlations between these parameters and the doses and blood concentrations of the drugs the duration of treatment seizure control etc Preliminary results from the folate analysis are presented

The material consists at present of 29 children (4–19 years old mean 11 years) treated for more than 3 years with fenytoin alone or together with other anti-epileptic drugs Serum folate levels below 5 ng/ml in 80% of the cases and below 3 7 ng/ml in 65% were found The lower folate levels correlated significantly with higher serum fenytoin concentrations There was no correlation to duration of treatment

Whole blood folate levels had a broad range and no significant decrease could be found 5 children had values below 20 ng/ml These 5 children had a higher total drug intake but the same fenytoin dose per kg bw compared with 7 children having whole-blood folate values above 40 ng/ml However the equal fenytion doses gave a higher serum fenytoin level in the children with the lower folate levels

A separate study of cabamazepin-treated children (as sole drug or additional) revealed the same findings large range and in some cases low values

Hb-values and serum B_{12} levels were normal in all cases

It is concluded that low serum folate values are common in children treated with fenytoin for more than 3 years the concentrations varying inversely with serum drug level Some children also have low total blood folate levels The correlation between other anti-epileptic drugs and folate levels can at present time not be set

G Blennow & N W Svenningsen *Lactate and pyruvate in cerebrospinal fluid in febrile convulsions*

The risk of epilepsy caused by cerebral hypoxic injury in febrile convulsions has earlier been pointed out in the literature The lactate pyruvate ratio is a sensitive indicator of cerebral hypoxia Lactate and pyruvate were therefore determined in the cerebrospinal fluid (CSF) from 14 children with febrile convulsions All the children had general convulsions of 1–20 min duration In two cases the convulsions were relapses (second and fourth episode) Neurological examination and psycho-motor development were normal in all cases as were protein content and cell counts in CSF The EEG was normal in all but one case this child having subcortical epilepsia

In all cases the lactate (mean 1 600 mmol/ml range 1 322–2 375 mmol/ml) and pyruvate (mean 0 115 mmol/ml range 0 084–0 192 mmol/ml) concentrations as well the ratio (mean 14 3 range 11 6–19 7) were within normal limits Neither could any

correlation to the time interval between convulsions and lumbar puncture be found

The results indicate that there is no severe cerebral hypoxia in febrile convulsion However the material is small and further investigation is in progress in children with this and other types of convulsions.

H. Ahlström *Pulmonary mechanics in infants after artificial ventilation A preliminary report*

Pulmonary symptoms are common in infants treated with mechanical ventilation in the neonatal period. The symptoms have been ascribed to oxygen toxicity mechanical damage or the pulmonary disease per se

A standardized technique including computerized calculations has made possible the routine investigation of pulmonary mechanics in infants Twelve infants treated with mechanical ventilation in the first month of life were studied 5 with idiopathic respiratory distress syndrome 4 with respiratory insufficiency syndrome and 3 with postasphyxia syndrome The infants were artificially ventilated for 9–463 hours with a mean oxygen concentration of 30–60% No infant got oxygen at concentrations higher than 80% The mean age for the first investigation was 7 weeks All but one infant were at the time of the investigations free from pulmonary symptoms One infant mechanically ventilated for 19 days had intercostal retractions during the first year of life but improved later Pulmonary X-ray showed in this case during the first months changes as in bronchopulmonary dysplasia.

Tidal volume breathing frequency and minute ventilation were normal in all infants Dynamic compliance was normal in 9 infants. It was low in those 3 infants mechanically ventilated for 200 hours or more. At reinvestigation 4 months later 2 of them were normal The infant who still had symptoms at the age of 10 months had a low compliance Pulmonary conductance was within the normal range in the first investigation but decreased generally during the subsequent months.

These results indicate that artificial ventilation for more than 1 week in a newborn baby may cause a reversible stiffening of the lungs independent of underlying disease even if comparatively low oxygen concentrations are used The decreasing pulmonary conductance during the first year indicates a narrowing of the airways which may be related to the tendency to obstructive disease in infants mechanically ventilated in the neonatal period The cause of the obstruction is unknown.

A. S Aronson S Garwicz & L Håkansson. *DNA synthesis in the peripheral blood in acute leukemia of childhood*

Acute lymphoblastic leukemia is often accompanied by lymphoblasts in the peripheral blood. A low percentage of blasts early during relapse might be very difficult to discover by routine differential count of blood cells A certain proportion of the blasts ought to synthesize DNA Thus these cells will be labelled if they are allowed to incorporate tritiated thymidine The presence of labelled cells can then easily be determined by liquid scintillation In total 11 children with acute lymphoblastic leukemia were studied in different phases of their disease

Ten patients in remission were studied in 12 test situations the range of the activity being below 290 cpm Four children in remission showed higher cpm values range 475–4702 due to pronounced proliferation of myelopoietic cells strong monocytosis or release of erythroblasts into the peripheral circulation

Five children in relapse had in 15 test situations cpm values ranging from 456 to 25444 (mean 5473) whereas 2 other patients in bone marrow relapse had low cpm values 113 and 127 respectively

When patients had high cpm values the effect of cytostatic treatment could be fol lowed using daily examinations Rapid decrease of cpm values indicated favourable response which could later on be confirmed by bone marrow remission

Often relapse during therapy could be detected early by the rise of DNA synthesi zing cells in the peripheral blood

In conclusion we have found that the pres ent method could help to validate remission identify relapse and disclose the therapeutic response to treatment

P O Petersson (Copenhagen) *Evaluation of mother and child health services in Europe*

During the last decade there has been an in- creasing interest in many European countries for evaluation of public health services

Services for mother and child health care offer a very good opportunity to study the trends during the last 10 years in most European countries since these services have been well developed at central regional and local levels

Based on available information countries were grouped according to the general struc ture of maternal and child health (MCH) services Each country was given a score on each of two scales—one scale describing the trend towards centralization the other one representing the degree of governmental versus private responsibilities in providing MCH services Based on this countries were divided into three groups Group A where MCH services were mainly a governmental responsibility Group B an intermediate group and Group C with a high degree of private MCH services As could be expected most countries in Group A had well-devel oped public health services for mothers and children

A trial to construct a quality index was done in assuming that the quality of a particular service increases with the number of activities implemented in the service and in addition that the number of subjects having access to and using these services also reflects the quality of the service Using such a quality index keeping in mind that this is a none too-accurate method it could be shown that countries in Group A tend to score higher than countries in Group C However there was considerable overlapping.

A quality index for prenatal and perinatal services was also compared with the figures for perinatal mortality Countries with a higher perinatal quality index tend to show lower perinatal mortality figures

In comparing the situation in 1960 with that in 1970 it could be shown that public health services are being made available to increasing numbers of mothers and children. This is especially the case in specific fields, such as for instance family planning ser vices for mothers

G Wettrell K. E Andersson Å Bertler & N R. Lundström *Concentrations of digox- in in plasma urine and tissues in infants and children with heart disease*

In order to find a safe and effective dosage of digoxin plasma levels of the glycoside were determined in 51 digoxin-treated infants and children aged 3 days to 10 years Digi talization was undertaken because of heart failure secondary to congenital heart defects or because of paroxysmal supraventricular tachycardia In infants less than 4 weeks of age the initial oral digoxin dosage was 0 05 mg digoxin/kg b wt Between 4 weeks and 1 year and between 1 year and 10 years of age 0 07 mg/kg b wt and 0 05 mg/kg b wt were administered respectively The daily mainte nance dose was 1/4 of the initial dose and was given in two equal amounts every 12 h Venous blood samples were collected during maintenance therapy between 10 and 12 h after the previous digoxin administration Plasma digoxin concentrations were deter mined by radioimmunoassay technique On comparable maintenance doses (0 012–0 013

mg digoxin/kg/day) the mean value of plasma digoxin was 1 2±0.5 (S D) ng/ml for infants ($n=10$) and 1 4±0 4 ng/ml for children ($n=17$) With the same daily dose of the glycoside a mean value of 2 1±0 6 ng/ml was obtained in neonates ($n=9$) In infants plasma digoxin concentrations increased from 1.2±0.5 ng/ml to 2.1±0 7 ng ($n=15$) when the dosage of the glycoside was increased from 0 012 to 0 019 mg digoxin/kg/day No clinical or electrocardiographic signs of toxicity were noted

Urine was collected for 24 h in 11 patients aged 1 week to 4 months on maintenance therapy with digoxin The digoxin content in the urine was determined by a modified ^{86}Rb-method. It was found that the renal clearance of digoxin was low during the first month of life but then increased with increasing age. An apparent correlation existed between renal clearances of digoxin and creatinine

In 3 patients on maintenance therapy the levels of digoxin in several tissues were determined postmortem by means of the ^{86}Rb-method High concentrations of the glycoside were found in kidney liver and heart A remarkably high digoxin concentration (>3× that of the heart) was found in the choroid plexus of the one patient where this tissue was examined This finding is of interest as a reduced production of cerebrospinal fluid during digoxin intake has been reported

Thus the plasma digoxin levels in infants and children achieved with the dose schedules used are in the same range as those associated with optimum clinical effect in adults. In neonates however higher values are found probably owing to a low renal clearance of glycoside It is not known whether this high digoxin level is required for an optimum inotropic effect on the myocardium of the neonate

G W Meeuwisse

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example is c.d.h. which, thanks to prophylaxis, has almost disappeared in Sweden.

In view of the above tendencies good comprehensive textbooks on pediatric orthopaedics are invaluable. Sharrard published an excellent textbook in 1971. In 1972 Mihran O. Tachdjian of Children's Memorial Hospital, Chicago published his Pediatric Orthopaedics (Saunders 1972), which is a welcome new-comer. The work consists of two volumes and has almost 2 000 pages. Tachdjian's book is a substantial, costly work. It gives detailed, well written information on the various diseases belonging to the realm of orthopaedic diseases in children. But it is also a surgical textbook and in the preface the author states that his original intention was to write a book on surgical techniques. This he found difficult to do without considering the biological principles of surgery, the dynamics of trauma, etc. The result was therefore a combined textbook, however, with a personal selection of surgical methods available. Thus, Harrington's operation for example, is not described in the chapter on idiopathic scoliosis.

The illustrations are of excellent quality, nearly all original and have been drawn by excellent medical artists. Some of the figures are redrawn from other works. In some cases one has the impression that the author has been over-ambitious in his illustration of the book. Technically relatively simple operations are illustrated in several stages, e.g. curettage and bone grafting of unicameral bone cyst in upper humerus, and excision of osteochondroma from medial aspect of distal femoral metaphysis. Five plates illustrating different stages from A to Q depict an infrequent operation of obstetric paralysis of the arm (modified Sever L'Episcopo Procedure). Moreover, one and the same plate is sometimes given a second time in a different part of the book, instead of referring to the first plate. This must have increased the costs of the book.

The book is very informative and readable. The introduction with the appendix of somewhat more than 60 pages is complete and contains everything about history taking, physical examination, neurophysiologic maturation levels (well illustrated), age of ossification of the different epiphyses etc.

In Congenital Deformities, most space is justly given to c.d.h. with excellent survey and good plates, e.g. Open reduction of c.d.h. including Salter technique and derotation osteotomy. No references are given in the chapter on congenital abduction contracture of the hip and pelvic obliquity and I have not been able to find this condition described in other textbooks.

In the description of Legg-Perthes disease the author refers also to personal investigations of intra-articular pressure of the hip in children with acute non-specific synovitis and stress—probably justly—that such increased pressure may be the cause of Legg-Perthes disease. In the treatment the author also describes a good orthosis, namely trilateral socket hip abduction orthosis.

In contrast with the impression given by other text books the author claims that Charcot Marie Tooth atrophy is not always a benign condition if defective gene is supplied by each parent.

The chapter on fractures (220 pages) is also detailed and praiseworthy. In the evaluation of the prognosis of femoral neck fractures Pauwel's angles are, as a rule, fairly difficult to assess depending on how roentgenograms are exposed. Like other British and American authors Tachdjian warns against the use of Bryant's traction for femoral shaft fractures in children older than two years because of the risk of ischaemic contracture. The author prefers ninety-ninety skeletal traction and other methods of traction.

The book contains well designed tables giving differential diagnoses and different methods of treatment, e.g. tables on tendon transfers for paralytic deformities of the foot and ankle and on differential diagnoses of various conditions with vitamin D deficiency and on differential diagnoses of the principal types of muscular dystrophy etc.

The extremely detailed bibliographic documentation for each disease in each chapter is impressive: 1206 references to the neuro-muscular system, including 300 for poliomyelitis, 146 references for talipes equinovarus, 88 for tarsal coalition and 36 references from three centuries for pulled elbow. Many of the orthopaedic theses published in Scandinavia are included in these lists. These references show that the author is extremely well-read.

The book is an extremely good contribution to our knowledge of pediatric orthopaedics. After reading Sharrard's textbook one can hardly help being more and more convinced that in several countries pediatric orthopaedics should be a subspecialty of orthopaedic surgery. The author is to be congratulated on completion of such a work which deserves a place on the shelf in the library of every orthopaedic and pediatric department.

Anders Hulth

C. F. Ferguson & E. L. Kendig Jr (eds.): *Pediatric Otolaryngology.* W. B. Saunders Company Ltd Publishers, London 1972, Vol. 2, 2nd ed. 517 pp. Illus. £10.65.

In this volume 41 contributing authors, all wellknown authorities, present the whole field of otolaryngology in children. Disregarding the fact that there is some overlapping of material, a distinguished and representative survey of advances in pediatric otolaryngology during the past two decades is presented.

The book is commendable.

Sven Ingelstedt

BOOK REVIEWS

K. Diebold *Die erblichen myoklonisch-epileptisch-dementiellen Kernsyndrome* In Hippius Janzarik & Müller (eds): Monographien aus dem Gesamtgebiete der Psychiatrie Psychiatry Series Band 8. Springer Verlag Berlin Heidelberg New York 1973 254 pp illus US $36 30

Dr Diebold is a German psychiatrist who has performed an extremely detailed study of some syndromes in which myoclonic fits are a main feature The syndromes studied Progressive Myoklonus-epilepsien—Dyssynergia cerebellaris myoclonica—myoklonische Varianten der drei nachinfantilen Formen der amaurotischen Idiotie are mentioned in the subtitle but the area covered by the book is not apparent from its main title From his study of the literature the author tries to define the term myoclonus and its pathophysiological background He seems to have found every case reported in the literature between 1800 and 1970 and gives a short description of them His own material collected from departments of neurology psychiatry and pediatrics in Germany and to some extent also in Austria and Switzerland is reported in a more sketchy way Apparently case records were analysed and living patients and their relatives were thoroughly examined clinically and electroencephalographically The only table in the whole book gives a survey of the number of patients examined personally by the author All other data about them are mixed with the cases from the literature to such an extent that it is impossible to differentiate between the author s own material and the literature cases. The author s own material is given in case reports covering almost 70 pages where each individual case is described with all details but it is nowhere summarised and discussed in relation to the literature reports The whole material is then divided according to histopathological clinical and neurophysiological findings The author finds two types of progressive myoclonus epilepsy with autosomal recessive inheritance and characterized histopathologically by intracellular accumulation of mucopolysaccharides so-called Lafora bodies in central nervous system liver skin muscles. The most common the main form of Lafora, usually had its onset at puberty with grand mal seizures and myoclonic jerks later followed by progressive demens and other neurological symptoms and signs and death within one decade The rare late type had a later onset and more prolonged course Another group consisted of cases of progressive myoclonus epilepsy in which the main histopathological features were degeneration of extrapyramidal and cerebellar neuronal systems without Lafora bodies, within this group cases with probably autosomal dominant inheritance and cases with probably autosomal recessive inheritance could be found In the cases labelled dyssynergia cerebellaris myoclonica the main histopathological features were atrophy of the dentate and superior cerebellar peduncles or degeneration of other cerebellar neuronal system both dominant and recessive inheritance could be found In the cases labelled myoclonic variants of the 3 postinfantile types of amaurotic idiocy neither clinical nor histopathological features distinguished them from cases of these syndromes without myoclonic jerks chemical and enzymatic aspects were not discussed

The book contains an enormous amount of information important to both pediatrician and neurologist. The reference list contains about 600 titles, mainly older literature about 10 are from 1969 and about 5 from 1970 or later However this rich material is presented in a way unkind to the reader Page after page is filled with details but no surveys summaries or conclusions are given. The lack of tables and illustrations is also distressing. One wishes that the author would use his valuable material to write a shorter book with less detail and presented in a more comprehensive way thus encouraging the ordinary lazy reader to find the fascinating knowledge that Dr Diebold has collected

Ingrid Gamstorp

M O Tachdjian. *Pediatric Orthopedics* W B Saunders Company Ltd London 197? Vol 1 766 pp illus., Vol. 2 1001 pp illus £27 65

Orthopaedic surgery has developed to a speciality comprising not only classical orthopaedics but also skeletal trauma. The increase in the number of orthopaedic clinics has resulted in a corresponding decrease in the population catered for by individual departments This has in turn resulted in a decrease in the specialist s experience of less common diseases. Many diseases in pediatric orthopaedics are relatively rare An extreme

Acta Pædiatr Scand 63 485–493 1974

CHILD HEALTH IN SWEDEN

STIG SJOLIN and BO VAHLQUIST

From the Department of Paediatrics University H spital Uppsala S eden

ABSTRACT Sjölin, S. and Vahlquist, B (Department of Paediatrics, University Hospital Uppsala, Sweden). Child health in Sweden. Acta Paediatr Scand 63: 485, 1974.—On the basis of relevant ital statistics, some characteristic features of the health of Swedish children during the last two centuries are presented and analysed with regard to probable causative factors. An attempt is also made to define in brief the child health problems of the future

KEY WORDS: Child health, child mortality child morbidity Sweden

Population Dynamics

Sweden has had its present boundaries since 1814 In the intervening years migration of population groups over the frontiers has at times materially influenced the demographic situation In the late 19th and early 20th centuries emigration dominated and after World War II, immigration. The net migration over the last three decades has resulted in a population increase in the order of half a million

The main forces however influencing the population dynamics of Sweden have long consisted in a successive decline of both the mortality and fertility rate (Fig. 1) For a period in between World War I and II there was a near balance between the two which meant that the endogenous population growth came to an almost complete standstill

A decline of the birth rate was observed already in the mid 19th century long before any systematic contraceptive methods were known to exist Since the beginning of the present century the tendency has gained momentum By this decline together with recent fluctuations in birth rate and migration the traditional population pyramid has been replaced by progressively more bizarre structures (Fig. 2)

Today the number of children below 15 years of age in Sweden constitutes only 21% of the whole population The proportion of persons aged 65 years or more on the other hand is as high as 14% The mean life expectancy for a newborn in Sweden today (1966–70) is 76 6 years for women and 71 9 for men (9)

Mortality in Childhood

Infant mortality figures are rightly looked upon as a sensitive indicator of the socioeconomic and health standards of a country In Sweden national figures for infant mortality have been available since 1749 and onwards, longer than in any other country In the early 19th century infant mortality was still about 200 per thousand (Fig. 3) Since about 1810 a gradual mostly steady decline has taken place leading down to the low figure of 11 1 per thousand in 1971

Boys run a greater risk than girls the latest figures (1971) for infant mortality for boys being 12 6 per thousand and for girls 9.3

ANNOUNCEMENTS

ASSOCIATION FOR PEDIATRIC EDUCATION IN EUROPE

The Association for Pediatric Education in Europe will have its Annual Meeting in Geneva on July 5 (beginning afternoon) 6 (a m and p m) and 7 (finishing lunch time)

In addition to the free papers there will also be discussions on the following three subjects

1 The responsibility of the pediatrician for the education of health personnel

2 The contents of the undergraduate pediatric curriculum

3 Teaching European medical students and doctors child health problems of developing countries

For additional information write to the General Secretary of APEE Spyros Doxiadis, Aghia Sophia Children s Hospital Athens 608 Greece

TAGUNG DER DEUTSCHEN GESELLSCHAFT FÜR KINDERHEILKUNDE 1974

Die *71 Tagung der Deutschen Gesellschaft für Kinderheilkunde* wird in der Zeit vom 9–11 September 1974 in Hamburg stattfinden Als Hauptthemen sind vorgesehen

I Pränatale Diagnostik und Therapie

II Überernährung – Unterernährung im Kindesalter

III Medizinische Aspekte der Adoleszenz

Weitere aktuelle Fragen aus den Gebieten pädiatrischer Forschung, Klinik und Praxis sollen in Vorträgen Symposien und Podiumsdiskussionen verhandelt werden Gemeinsame Sitzungen mit den Deutschen Gesellschaften für Kinderchirurgie für Pädiatrische Kardiologie und für Sozialpädiatrie sind vorgesehen

Die Tagung soll unter dem Vorsitz von Prof Dr K H Schäfer Hamburg 20 Martinistr 52 stattfinden.

ERRATA

In the article by U N Wiesmann et al Mucolipidosis II (I-cell disease) which appeared in the January issue of Acta Paediatrica Scandinavica (63 9–16 1974) lines 4–11 right column page 15 should read as follows. Heat inactivation and pH optimum of the enzymes were normal and therefore did not suggest any abnormality in the structure of the enzyme protein The administration of drugs known to stabilize lysosomal membranes *in vivo* had no effect on the elevated enzyme levels in the plasma of the patient except for high doses of prednisone which resulted in a further increase in the enzyme activity

In the article by Carl Johan Edeling ^{99m}TC Pertechnetate brain scintigraphy in children *Acta Paediatr Scand 63* 193–200 1974 the following lines should read

p 196, left column, lines 6 and 7 Two scintigrams were positive because of burr-holes. Positive scintigrams were obtained

p 196 right column lines 25 and 28 *Scintigraphy* In the anterior view (Fig 2 *a*) there is an *and* region on the right lateral scan (Fig *b*).

p 197 left column lines 13 and 16. In the frontal region In the left lateral view (Fig 3 *a*) there *and* (Fig. 3 *b*) there is abnormal activity in both hemispheres

p 198 right column line 6 ^{203}Hg-chlormerodrine and ^{131}I HSA Seven

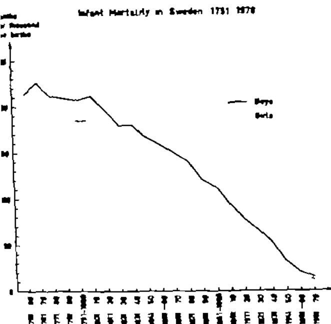


Fig 3 Infant mortality in Sweden, 1751–1970 (2, 9).

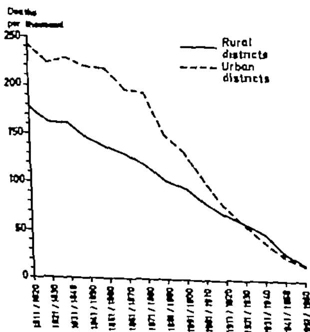


Fig 4 Infant mortality in rural and urban dist in Sweden, 1811 1960 (2).

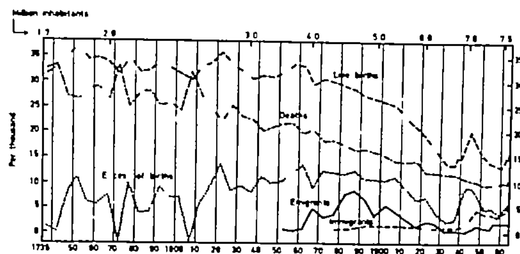


Fig 1 Population changes in Sweden 1735–1965 Per thousand mean population (?)

Until about 1930 infant mortality was higher in urban than in rural districts and for the next few decades the opposite held true (Fig 4) Nowadays no difference exists It is also of interest to note that infant mortality was once much higher in the sparsely populated areas of northern Sweden than in other parts of the country In 1936–40 for instance the county of Norrbotten around the arctic circle with 250 000 inhabitants and a population density of about 3 per sq km had an infant mortality of 65 per thousand while the figure for the county of Uppsala was 35 This difference has now been eliminated (Fig 5)

The decline in infant mortality covers all parts of the first year of life Perinatal mortality which was for a long time rather refractory has also shown an encouraging decline during the last 30 years from 45 per

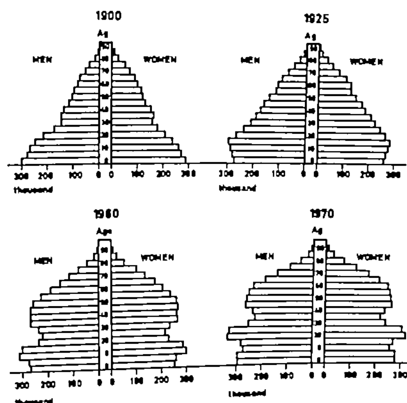


Fig 2 Age pyramids in Sweden 1900 1925 1960 and 1970 (?)

Table 3 *Leading causes of death per 100 000 children in different age groups over 1 year in Sweden in 1911–1915 and 1966–1970 (A M Bolander)*

	Age group					
	1–4 years		5–9 years		10–14 years	
Period	1911–15	1966–70	1911–15	1966–70	1911–15	1966–70
Infectious diseases	245	3	91	1	46	1
Diseases of the respiratory system	190	6	28	3	14	1
Tuberculosis	143	0	77	0	96	0
Diseases of the digestive system	65	2	18	1	13	1
Unknown	63	0	18	0	10	0
Accidents	45	18	26	17	4	1
Congenital anomalies	17	0	1	0	0	0
Tumours	4	10	3	8	3	6
Others	106	17	56	8	60	7
Total	878	56	318	38	266	28

see a child with nutritional anaemia, rickets or goitre Tuberculosis in children diphtheria and polio have been virtually eradicated Other infectious diseases such as tetanus salmonellosis and also whooping cough are very uncommon. Diarrhoeal disease still represents a problem in young children although much less so than it did only a few decades ago Mild and commonplace diseases such as upper respiratory infections allergic diseases etc are as frequent as ever

Table 4 shows the disease pattern commonly encountered in a paediatrician's consulting room Only 3% of the patients represented in the table had to be referred to a hospital The relative frequencies of the most common diagnoses among hospitalized children in the Uppsala region can be seen in

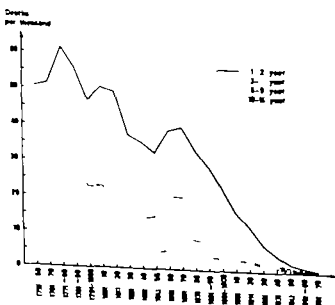


Fig 7 Mortality among children aged 1–14 years in Sweden, 1951–1970 (2, 9)

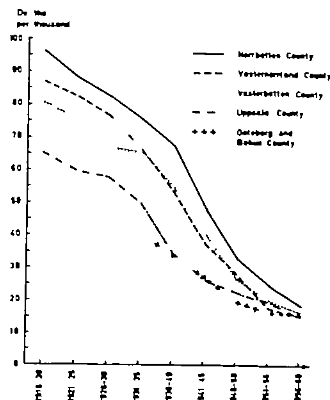


Fig 5 Infant mortality in five Swedish counties 1916–60 (Department of Social Medicine University of Uppsala)

thousand in 1940 to 16 per thousand in 1970 (Fig. 6) Already after the first week of life there is a steep decline in mortality nowadays (Table 1)

In older children too there has been a marked fall in mortality since the early 19th century and since about 1870 the fall has been continuous (Fig 7) The latest mortality figures for older children are very low (Table 1)

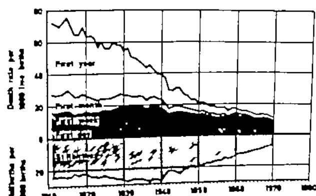


Fig 6 Infant and perinatal mortality in Sweden 1910–70 (7 9)

Table 1 *Child mortality in different age groups per thousand in Sweden in 1970 (9)*

Age period	Mortality
0–6 days	8.1
7 days to 11 months	2.9*
1–4 years	0.53
5–9 years	0.41
10–14 years	0.28

* Per thousand live births.

Main Causes of Death in Childhood

The pattern of causes of death varies markedly with age group as is evident from Tables 2 and 3 The main killers of children today are very different from those only a few decades ago When the preventable diseases (malnutrition many infections etc) have been brought under control there remains a hard core of refractory killers which are much more difficult to come to grips with (some diseases of the newborn malformations malignant conditions and accidents)

Morbidity in Children

The disease pattern among children in Sweden today shows hardly any striking deviations from that observed in other European countries with a similar standard of living Basically preventable diseases such as those caused by nutritional deficiencies and infections are under good control It is very rare today to

Table 2 *Leading causes of death per 100 000 children below 1 year of age in Sweden in 1911–15 and 1966–70 (A M Bolander)*

	1911–15	1966–70
Diseases of the newborn	2 890	750
Congenital anomalies		300
Diseases of the respiratory system	1 700	40
Diseases of the digestive system	900	30
Infectious diseases	710	20
Tuberculosis	200	0
Accidents	1 320	70
Other causes		10
Total	7 720	1 220

Table 3 *Leading causes of death per 100 000 children in different age groups over 1 year in Sweden in 1911–1915 and 1966–1970 (A M Bolander)*

	Age group					
	1–4 years		5–9 years		10–14 years	
Period	1911–15	1966–70	1911–15	1966–70	1911–15	1966–70
Infectious diseases	45	3	91	1	46	1
Diseases of the respiratory system	190	6	28	3	14	1
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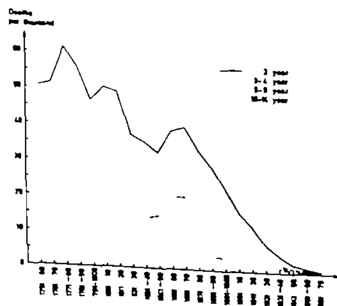


Fig 7 Mortality among children aged 1 14 years in Sweden, 1951 1970 (2, 9)

Table 4 *Relative diagnosis frequencies among first visits to a paediatric practitioner in Stockholm (6)*

	Percent
Upper respiratory infections	[illegible]
Otitis media	20*
Psychosomatic diseases	14
Infectious fevers	8
Allergic diseases	5
Urinary tract infections	3
Acute gastrointestinal diseases	2
Pneumonia	2
Others	14
Total	100

* This figure may be above average since the practitioner in this case had a special ENT training

Table 5 It should be added that recent comprehensive studies on the health of 4 year-old children revealed a remarkably high incidence of caries (74–83%) and also showed that dental health was correlated to the socio-economic situation of the parents (3 7) Another health problem of today revealed by these studies of 4 year-old children is the strong need felt by many parents to obtain better information concerning child rearing and more help with their children s behavioural disturbances (4 11)

Table 5 *Relative frequencies of the most common diagnostic groups in hospitalized children aged 0–14 years in the Uppsala region with 1.2 million inhabitants in 1969*
International Statistical Classification of Diseases WHO 1965

	Percent
Diseases of the respiratory system	26.5
Accidents poisoning and violence	10.5
Symptoms and ill-defined conditions	9.6
Infective and parasitic diseases	8.5
Diseases of the nervous system and sense organs	7.9
Congenital anomalies	7.5
Diseases of the digestive system	6.8
Diseases of the newborn	6.3
Diseases of the genito-urinary system	4.9
Diseases of the blood	2.0
Others	9.5
Total	100.0

Organization of Hospital and Health Care

The number of doctors paediatricians and registered nurses in relation to the population, as well as the number of hospitals with paediatric departments and of child health centres are given in Table 6

A new plan has recently been adopted by the National Board of Health and Welfare which means that the primary medical care of children as a rule should rest with fully qualified district paediatricians

SWEDISH CHILD HEALTH PANORAMA OF TODAY

Positive features

Low perinatal and infant mortality rates An important factor in both is undoubtedly the comparatively low prematurity rate (ca. 4% of all newborns weigh less than 2500 g)

An adequate number of hospital beds for children is available all over the country

Regular health check up for practically all children during the first 2 years of life at the public health centres (Table 6) After that age a decline in attendances is often noted but this is counterbalanced by a thorough check up at the age of 4 years which includes evaluation of the behavioural pattern testing of vision and hearing and dental examination

Nutrition is well cared for in the early years and extensive use is being made of commercial formulas and baby food of high standards (ca. 500 jars of baby food consumed per infant and year!) Basic information for health personnel and parents about child nutrition has recently been published by the National Board of Health and Social Welfare

Immunization schemes well covering the childhood population Vaccination against tuberculosis smallpox diphtheria whooping cough tetanus polio and recently also measles are included

The care of chronically ill and handicapped children is well organized This is true for children with mental retardation severe be

Table 6. *Medical personnel and some health facilities for children in 1970 (1)*

No. of doctors	10950
per 1000 inhabitants	1.35
No. of paediatricians	443
per 1000 children 0–14 years	0.26
No. of nurses	32700
per 1000 inhabitants	4.1
No. of paediatric departments	44
No. of paediatric beds	2566
per 1000 children 0–14 years	1.5
No. of child health centres	1259

No. of supervised 1-year-olds 99%
No. of supervised, 2-year-olds 98%
No. of supervised 2–7-year-olds 67%

havioural disturbances cerebral palsy impairment of vision and hearing, diabetes cardiac disease cystic fibrosis, phenylketonuria etc. However the care of children with asthma and epilepsy is still not entirely satisfactory

Health education of parents is not systematic but reasonably well covered and practised on an individual basis during visits to child health centres and especially at regular home visits by nurses

Social and economic benefits of various kinds are on the whole well developed for families with small children especially low income families For details see Trygg Hansa *Social benefits in Sweden* (13)

Negative features

Shortage of paediatricians For the time being only about 400 are active For realization of the scheme for coverage of all rural as well as urban areas by district paediatricians at least another 500 are needed

Shortage of kindergartens and day-care centres The number is rapidly increasing but has not kept pace with a fast-growing demand (many more women engaged in work outside their homes)

Decline in breast feeding A recent study in Uppsala has indicated that only 17% of the infants are still completely breast-fed at 3 months The lax attitude to breast feeding (not only on the part of mothers but also of doctors and nurses) which is indeed found in many countries today may meet a challenge in the near future

Unsatisfactory attention to mental health problems at the child health centres Child psychiatry is well developed each one of the 25 provinces having at least one department of its own. Efficient primary care of children with developmental disturbances or signs of maladjustment requires however paediatricians with better training and more time for this kind of work Likewise an increased number of positions for child psychologists working in collaboration with doctors and nurses of child health centres is sorely needed

School health organization needs considerable strengthening and partly reorganization. The need to recruit school child psychiatrists on at least a part time basis is becoming more pressing as tendencies to severe emotional disturbances juvenile delinquency and addictions of various kinds are increasing in frequency even in younger age groups

Difference in accessibility of health services between urban and rural areas Much improvement is still needed as regards the accessibility of health services in rural areas In many cases for example the distances between homes and child health centres are much too great A change for the better is expected when the new district paediatrician programme comes into operation

COMMENTS

The greatly improved physical health of Swedish children as reflected in the marked fall of mortality in all age groups raises the important question of causative factors Two features of the changing pattern of child health deserve particular attention when analysing the cause–effect relationship One is the almost continuous uninterrupted decline in infant and child mortality from 1810 (Figs 3 and 7) and the other is the steady decrease in perinatal mortality since 1940 (Fig. 6)

As no reliable statistics concerning causes

Table 4 *Relative diagnosis frequencies among first visits to a paediatric practitioner in Stockholm (6)*

	Percent
Upper respiratory infections	3[illegible]
Otitis media	[illegible]0*
Psychosomatic diseases	14
Infectious fevers	8
Allergic diseases	5
Urinary tract infections	3
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Pneumonia	
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Total	100

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International Statistical Classification of Diseases WHO 1965

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Accidents, poisoning and violence	10 5
Symptoms and ill-defined conditions	9 6
Infective and parasitic diseases	8.5
Diseases of the nervous system and sense organs	7 9
Congenital anomalies	7 5
Diseases of the digestive system	6.8
Diseases of the newborn	6.3
Diseases of the genito-urinary system	4 9
Diseases of the blood	[illegible]0
Others	9.5
Total	100 0

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of death are available for the years before 1910 it is not possible to establish with certainty the causes of the falling infant mortality during the 19th century There is however no reason to believe that medicine itself played any appreciable role in this early development It seems far more likely that the health of children was affected favourably by more unspecific changes taking place in society at that time While the general economic expansion and advancements in the standard of living and education constituted a firm basis for the improvements in health improved nutrition was probably the most direct and most important cause of the decreasing mortality

At the end of the 19th century the major discoveries in the field of bacteriology gave rise to a series of sanitary measures that were to prove of utmost significance for the health of the people These measures were designed as a rule for the whole population but it was often the children in particular that gained from them

The continuous improvement in these two factors nutrition and sanitation prevailed as the dominant cause of the decline in mortality also during the first two decades of the 20th century It was probably not until the 1920s that clinical research contributed such new and effective means of preventing and treating physical disease that the mortality among children was influenced

In spite of the most impressive decrease in infant mortality as a whole it was observed for several decades in the 20th century that both the neonatal mortality and the still-birth rate remained uninfluenced by all social and medical progress During the 1930s there was even an increase in perinatal mortality as given in official statistics in Sweden (Fig. 6) The decline that started in 1940 must be regarded as a result of the intentional and joint efforts of obstetricians and paediatricians to combat death in late foetuses and newborn infants These efforts led to increased knowledge and to the development of new methods of treatment and care of pregnant women and their newborn infants

From these examples it seems justifiable to draw the general conclusion that good child health as evidenced by low mortality figures can be achieved in a country only by a combination of social economic and medical improvements and measures offered to all families and children

It is difficult to predict the lowest future figure for infant mortality but it would seem safe to say that we are now very close to it. In this context it is of interest to note that already during the years 1900–30 infant mortality among royal families in Europe was only 8 per thousand (12)

Finally it should be stressed that for a long time child mortality figures very accurately reflected the major and most overwhelming paediatric problems and at the same time comprised a useful guide for the planning of the future care of sick and healthy children This is no longer the case however The very low mortality figures in Sweden today give only limited information as to the major health needs of children In order to obtain a more revealing picture and a reliable basis for further development it is becoming more and more urgent to focus on the morbidity situation and the effects of disease on children. It also seems desirable to take into account more fully the mental health social adjustment and human needs of children

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Miss Anne Marie Bolander of the National Central Bureau of Statistics for her help in providing some of the statistical data

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of death are available for the years before 1910 it is not possible to establish with certainty the causes of the falling infant mortality during the 19th century There is however no reason to believe that medicine itself played any appreciable role in this early development It seems far more likely that the health of children was affected favourably by more unspecific changes taking place in society at that time While the general economic expansion and advancements in the standard of living and education constituted a firm basis for the improvements in health improved nutrition was probably the most direct and most important cause of the decreasing mortality

At the end of the 19th century the major discoveries in the field of bacteriology gave rise to a series of sanitary measures that were to prove of utmost significance for the health of the people These measures were designed as a rule for the whole population but it was often the children in particular that gained from them

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From these examples it seems justifiable to draw the general conclusion that good child health as evidenced by low mortality figures, can be achieved in a country only by a combination of social economic and medical improvements and measures offered to *all* families and children

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Table 1 *Code designation and age at death of the patients with Krabbe's disease*

Code designation: Hagberg et al. (10)	Code designation: Present study	Age at death (months)
A.W 60		7 1/2
	M.O 68	8
	M.-O C. 70	9
S.W 66		9 1/2
J W 67		12
	P.J 69	13
K R. 62		13
	A.R. 68	13
S B 61		15
P B 65		15
A.-K.Å. 66		15
K.J 61		16
K.W 62		19
E M. L. 59		20 1/2
	L.E. 69	20 1/2
	A J 70	25
A.H. 61		28
M S 60		32

pathology of Krabbe's disease have appeared (2, 5 6 7 8 11 13 15 18) no systematic study of the lipid changes has been performed on an appropriate material and the various research groups have obtained conflicting results (22) Understanding of the pathogenesis of Krabbe's disease requires a thorough knowledge of the biochemical changes in the condition, and we therefore undertook the present study

MATERIAL AND METHODS

Brain tissue

The material consisted of autopsy specimens of the brain from 18 cases of Krabbe's disease. The subjects had died at 7–32 months of age The diagnosis had been established from the clinical signs and symptoms, and pathological-anatomical examination of autopsy and/or biopsy material. In 4 cases (M.-O C. 70, P.J 69 L.E. 69 and A J 70) the clinical diagnosis was verified by the assay for cerebroside β-galactosidase (16) instead of by histological examination. The clinical records of 1 of the patients were included in a recent study of 32 Swedish cases (10) The code names and ages of the patients at death are given in Table 1 The control material consisted of 16 normal brains, from subjects aged 6–27 months (19). The cerebral cortex and white matter were dissected from the frontal lobe and kept at −20° until analysed.

Table 2 *Major brain lipids in cerebral cortex of patients with Krabbe's disease and of age-matched controls*

	Controls 6–27 months (n=16)		Krabbe's disease 7–32 months (n=18)		Significance level
	Mean	S.D	Mean	S.D	
Water*	86.0	1 7	83.8	1 0	
Total phospho-lipids	40 6	4.3	37.6	3.[illegible]	0 05
EPG	35 4	2.5	33.6	2.0	0.05
SPG	13 8	1.6	13 7	1.6	
IPG	[illegible]	0.9	3 1	0.5	0 01
CPG	34.3	0 9	34.6	1 4	
Sph	10 0	1.3	11 1	1 1	0 01
Cholesterol	24 0	3 6	23.8	2.7	
Lipid-NANA	[illegible]	0 23	2 08	[illegible]	0 05

Values are expressed in μmol/g wet weight, except for water (%) and individual phospholipids (mole %).

Abbreviations used. CPG choline phosphoglycerides EPG ethanolamine phosphoglycerides IPG, inositol phosphoglycerides. SPG serine phosphoglycerides Sph, sphingomyelins; NANA, *N*-acetylneuraminic acid

Methods

A total lipid extract was prepared in chloroform-methanol and freed from non-lipid contaminants by solvent partition (21). All quantitative lipid determinations and fatty acid analyses were performed in the same way as in normal human brains (19 20). Ethanolamine plasmalogens were determined in three cases with a method recently developed in this laboratory (L. Karlsson, personal communication). Myelin was isolated with a procedure designed by Norton (14) from 20 g of white matter from 2 patients (P.J 68 and A.J 70) from 8 g of white matter of a normal 4-month-old infant and from 4 g of white matter of a normal 12 month-old infant.

RESULTS

Cerebral cortex

The concentrations of the major lipid classes are given in Table 2. That of cholesterol was normal while those of total phospholipids and gangliosides expressed as lipid NANA were slightly reduced Small variations in the patterns of the phospholipids were also found in Krabbe's disease: ethanolamine phosphoglycerides were diminished, while sphingomyelins and inositol phosphoglycerides were increased Cerebrosides and sulfatides were

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CHEMICAL PATHOLOGY OF KRABBE S DISEASE

I Lipid Composition and Fatty Acid Patterns of Phosphoglycerides in Brain

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ABSTRACT Vanier M Th. and Svennerholm L. (Department of Neurochemistry Psychiatric Research Centre University of Göteborg Göteborg Sweden). Chemical pathology of Krabbe s Disease I Lipid composition and fatty acid patterns of phosphoglycerides in brain. Acta Paediatr Scand 63: 494 1974.—Lipid biochemical determinations were performed on autopsy material from 18 cases of Krabbe s disease aged 7–23 months, collected in Sweden between 1960 and 1972.

Cerebral white matter The lipid concentration of the cerebral white matter was found substantially reduced It was the same in 17 cases, independent of age at death and rather similar to that of cerebral cortex. All major lipids were diminished but the myelin lipids, cholesterol and particularly galactolipids (cerebrosides and sulfatides) were reduced more than the phospholipids. The ratio cerebroside/sulfatide was significantly increased but this was not a constant finding The relative percentage of ethanolamine phosphoglycerides was decreased and that of choline phosphoglycerides increased The fatty acid composition of the phosphoglycerides shifted towards values found in the white matter of 2 to 4 month-old infants. In addition a characteristic significant increase of arachidonic acid occurred.

Cerebral cortex. Only minor changes were observed but they were of the same type as some of the changes of the white matter a decrease in the relative percentage of ethanolamine phosphoglycerides and an increase of arachidonic acid.

The lipid changes in Krabbe s disease are suggested to be the result of the combined effect of a serious myelin deficiency and the replacement of the normal brain cytoarchitecture by proliferated astroglial cells, epithelioid cells and globoid bodies.

KEY WORDS Myelin lipid deficiency globoid cells, diminished sulfatide/cerebroside ratio, increased arachidonic acid concentration

Globoid cell leucodystrophy or Krabbe s disease is an inherited disease of the nervous system The primary metabolic lesion seems to be a deficiency of a β-galactosidase which hydrolyses the cerebrosides (galactosyl ceramides) of brain (16) This disease has been considered rare and in recent review Suzuki & Suzuki (17) found less than 100 cases on record The frequency of the disease seems to be particularly high in Scandinavia In a survey of the Swedish cases diagnosed during the period 1953–67 Hagberg et al (10) reported an incidence rate of 1 50000 It is the impression of the present authors that this figure is too low and that with better diagnostic methods it would be found higher Apart from the genetic study Hagberg s paper also gave a comprehensive description of the clinical signs and symptoms and the morphological features in Krabbe s disease Although several reports on the chemical

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Table 4 Major brain lipids in cerebral white matter of patients with Krabbe s disease and of age-matched controls

	Controls 6→27 months	Krabbe s disease 8–3 months (n = 18) Mean	S.D
Water*	81→77	84 0	4
Phospholipids	66→107	34 8	5 7
EPG	37→36	31 3	1.8
SPG	17→20	14 0	1 2
IPG	1–4	3 4	0 6
CPG	34→79	36 6	5
Sph	10→15	14 9	2 6
Cholesterol	56→110	4.3	7 0
Cerebrosides	13→37	3	1 5
Sulfatides	4→7	0 5	0 3
Lipid-NANA	1 4→0 9	1.51	0 38

Values are expressed in μmol/g wet weight, except for water (%) and individual phospholipids (Mole %)
For abbreviations, see Table 2

creased. The cerebroside/sulfatide ratio was significantly higher in the cases with Krabbe s disease (7 8 S D 3 9) than in the control group (4 1 S D 0 8) but the inter-individual variation was wide The pattern of the phospholipeds was also affected. The relative concentrations of CPG was increased and higher than in the youngest controls while those of EPG and SPG were lower than what was found in the same controls (6–7 months) Ethanolamine plasmalogens constituted approximately 45% of the EPG-fraction which is much less than in normal white matter (60–70%) and similar to the values in normal cerebral cortex. Their composition of dimethylacetals was also the same as in normal cerebral cortex except for a higher value for 18 1 dimethylacetal IPG were within the normal range as were sphingomyelins The relative concentration of this latter fraction was of the same magnitude as that found in 1–2 year-old controls

One case A.H 61 fell considerably outside the mean value and had relatively higher concentrations of phospholipids (45.5 μmol/g) cholesterol (39 5 μmol/g) cerebrosides (4 6 μmol/g) and sulfatides (1 6 μmol/g) with a low cerebroside/sulfatide ratio

The fatty acid compositions of EPG SPG and CPG are given in Table 5 The same types of changes were observed in all three

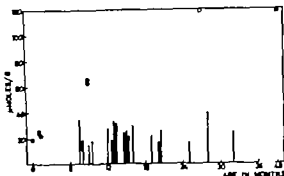


Fig 2 Concentration of cholesterol in cerebral white matter of normal infants (O) and of patients with Krabbe s disease (columns)



Fig 1 Concentration of phospholipids in cerebral white matter of normal infants (Δ) and of patients with Krabbe s disease (columns)

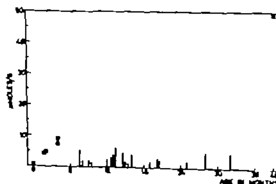


Fig 3 Concentration of cerebrosides in cerebral white matter of normal infants (■) and of patients with Krabbe s disease (columns)

Table 3. *Fatty acid composition of major phosphoglycerides in cerebral cortex of normal infants and patients with Krabbe's disease*

	EPG				SPG				CPG			
	Normals (n = 10)		Krabbe's disease (n = 11)		Normals (n = 10)		Krabbe's disease (n = 11)		Normals (n = 10)		Krabbe's disease (n = 11)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
16:0	5.9	0.6	6	0.9	3.9	1.1	4.8	1.1	47.8	1.0	48	1.5
16:1	0.7	0.1	0.6	0.2	0.5	0	0.5	0.2	3.5	0.4	2.9	0.7
18:0	27.9	3	27.1	2	43.0	6	41.9	2.5	12	0.8	10.9	1.0
18:1	8.9	1.1	9.0	1.0	8.9	1	10.1	1.6	4.9	1.1	25.7	1.1
20:3 (n-6)	1.4	0.5	1.7	0.4	1.6	0.2	1.7	0.4	1	0.3	1.2	0.3
20:4 (n-6)	17.4	1.9	19.0	1.3	3.1	0.5	4.3	1.1	6.2	1.4	7.2	1.2
22:4 (n-6)	11.7	2.1	10.6	1	7	1.3	6.7	1.0	0.9	0.3	0.8	0.1
22:5 (n-6)	5.0	1	5.3	2.5	7.9	3.6	7.6	3.4	0.4	0	0.4	0.2
22:6 (n-3)	19.1	3.3	19.3	4.0	6	3.6	1.0	4.4	1.6	0.4	1.4	0.6
Linoleic acid series	36.2	5.6	36.9	3.4	20.3	6.8	20.6	3.7	9	2.2	10.0	1.7
Linolenic acid series	19.6	3.3	19.7	4.2	22.9	3.5	21.4	4.6	1.6	0.4	1.6	0.4

Values are weight percentages of methyl esters. The following were detected in small amounts (in general less than 1%) and were added to the other values to make a total of 100%: 18:2 (n-6), 18:3 (n-6), 20:3 (n-9), 22:5 (n-3), 24:4 (n-6).

not determined in the individual cases because the concentrations were very low and depended on which layers of the cerebral cortex constituted the specimens. The fatty acid compositions were determined in the major phosphoglycerides. The values found for ethanolamine phosphoglycerides (EPG), serine phosphoglycerides (SPG) and choline phosphoglycerides (CPG) are reported in Table 3, while those of inositol phosphoglycerides (IPG) are not given because no such differences were found between the controls and the subjects with Krabbe's disease. The fatty acid compositions of the other three phosphoglycerides were also very similar in the controls and in the patients, and only small variations were observed: a decrease of 18:0 in CPG, and increase of 18:1 in SPG and CPG, and an increase of 20:4 (n-6) in EPG and SPG were found in Krabbe's disease.

Cerebral white matter

During the first 2 years of life, the lipid composition of the cerebral white matter normally undergoes large changes as myelination proceeds. The mean values and standard deviations were therefore not calculated for the control group. Instead, the values found in the youngest (6–7 months) and the oldest (25–27 months) normal brains are given and the alterations with age are indicated by an arrow. In the brains of the subjects with Krabbe's disease the lipid composition did not undergo any changes with age and the values could therefore be treated statistically. The lipid composition of white matter is reported in Table 4 and Figs. 1–3.

The water concentration was increased and reached values of the same magnitude as in cerebral cortex. The concentrations of all major lipids were strongly reduced (Table 4). The inter-individual variation was small and entirely independent of the age at death, which is clearly evident when the results for phospholipids (Fig. 1), cholesterol (Fig. 2) and cerebrosides (Fig. 3) are presented graphically. The concentrations of phospholipids were 53% of cholesterol 43%, of cerebrosides 25% and of sulfatides 13% of the values found in the youngest control brains (6–7 months). The gangliosides were in

Table 4 Major brain lipids in cerebral white matter of patients with Krabbe s disease and of age-matched controls

	Controls 6→27 months	Krabbe disease 8–32 months (n = 18)	
		Mean	S D
Water*	81→77	84 0	2 4
Phospholipids	66→107	34.8	5 7
EPG	37→36	31 3	1 8
SPG	17→20	14 0	1
IPG	1–4	3 4	0 6
CPG	34→29	36 6	5
Sph	10→15	14 9	6
Cholesterol	56→110	4 3	7 0
Cerebrosides	13→37	3	1 5
Sulfatides	4→7	0 5	0 3
Lipid-NANA	1 4→0.9	1 51	0 38

Values are expressed in µmol/g wet weight, except for water (%) and individual phospholipids (Mole %) For abbreviations, see Table

creased. The cerebroside/sulfatide ratio was significantly higher in the cases with Krabbe s disease (7.8 S D 3 9) than in the control group (4 1 S D 0 8) but the inter-individual variation was wide The pattern of the phospholipids was also affected The relative concentrations of CPG was increased and higher than in the youngest controls while those of EPG and SPG were lower than what was found in the same controls (6–7 months) Ethanolamine plasmalogens constituted approximately 45% of the EPG-fraction which is much less than in normal white matter (60–70%) and similar to the values in normal cerebral cortex Their composition of di methylacetals was also the same as in normal cerebral cortex except for a higher value for 18 1 dimethylacetal IPG were within the normal range as were sphingomyelins The relative concentration of this latter fraction was of the same magnitude as that found in 1–2 year-old controls

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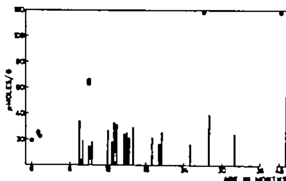


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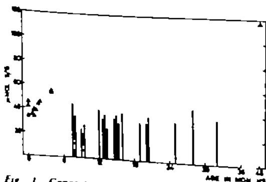


Fig 1 Concentration of phospholipid in cerebral white matter of normal infants (Δ) and of patients with Krabbe disease (columns)

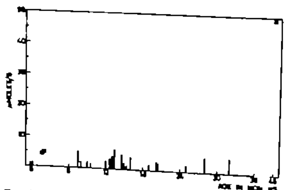


Fig 3 Concentration of cerebrosides in cerebral white matter of normal infants (■) and of patients with Krabbe disease (column).

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	EPG				SPG				CPG			
	Normals ($n=10$)		Krabbe's disease ($n=11$)		Normals ($n=10$)		Krabbe's disease ($n=11$)		Normals ($n=10$)		Krabbe's disease ($n=11$)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
16:0	5.9	0.6	6.2	0.9	3.9	1.1	4.8	1.1	47.8	1.0	48	1.5
16:1	0.7	0.1	0.6	0	0.5	0	0.5	0.2	3.5	0.4	2.9	0.7
18:0	7.9	3	27.1	2	43.0	6	41.9	[illegible]5	12	0.8	10.9	1.0
18:1	8.9	1.1	9.0	1.0	8.9	1.2	10.1	1.6	24.9	1.1	[illegible]5.7	1.1
20:3 (n-6)	1.4	0.5	1.7	0.4	1.6	0.2	1.7	0.4	1.2	0.3	1.[illegible]	0.3
20:4 (n-6)	17.4	1.9	19.0	1.3	3.1	0.5	4.3	1.1	6.2	1.4	7	1.2
22:4 (n-6)	11.7	1	10.6	1.2	7	1.3	6.7	1.0	0.9	0.3	0.8	0.1
22:5 (n-6)	5.0	1	5.3	2.5	7.9	3.6	7.6	3.4	0.4	0.2	0.4	0.[illegible]
22:6 (n-3)	19.1	3.3	19.3	4.0	6	3.6	1.0	4.4	1.6	0.4	1.4	0.6
Linoleic acid series	36	5.6	36.9	3.4	20.3	6.8	20.6	3.7	9.2	2	10.0	1.7
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The water concentration was increased and reached values of the same magnitude as in cerebral cortex. The concentrations of all major lipids were strongly reduced (Table 4). The inter-individual variation was small and entirely independent of the age at death, which is clearly evident when the results for phospholipids (Fig. 1), cholesterol (Fig. 2) and cerebrosides (Fig. 3) are presented graphically. The concentrations of phospholipids were 53% of cholesterol 43%, of cerebrosides 25% and of sulfatides 13% of the values found in the youngest control brains (6–7 months). The gangliosides were in

(18) reported studies on fresh material from 2 classical cases In the white matter there was a decrease of all lipids particularly of the myelin lipids Phospholipids were reduced to 50% and cerebrosides+sulfatides to 20–25% of the amount in age-matched controls The amount of sulfatides was particularly reduced The diminution of cerebrosides and the increased ratio of cerebroside/sulfatide was confirmed by Austin (1 2) and Austin & Lehfeldt (4) Eto & Suzuki (8) found in one case strongly diminished concentrations of cerebrosides and sulfatides but a normal ratio between them.

The present study has convincingly shown that the concentrations of cerebrosides and sulfatides are substantially reduced in Krabbe's disease and that no correlation can be demonstrated between their concentrations and the duration of the disease The cerebroside/sulfatide ratio was increased from 4 1 1 to 7.8 1 but the inter-individual variation was wide and in 6 cases out of 18 the ratio was not significantly increased In the myelin of 2 cases we found this ratio to be 3 0 1 and Eto et al. (9) also found a low ratio 2.7 1 in their case Austin (2) demonstrated that cases with more globoid bodies determined by histological methods tended to have higher cerebroside values Ours and Eto et al (9) finding of a low cerebroside/sulfatide ratio in myelin in cases of Krabbe's disease lends support to Austin's suggestion (2) that the increase of the cerebroside/sulfatide ratio is due entirely to the storage of cerebrosides in cells of mesenchymal origin

Brante (6) found that the proportions between the various main lipid groups in white matter have shifted towards those in grey matter as the result of the almost complete lack of myelin. In the present study the concentrations of cholesterol and phospholipids were very similar in white matter and cerebral cortex. However the lipid composition of cerebral cortex and that of white matter in Krabbe's disease were not identical with each other or with the cerebral cortex of normal brains The absolute amount of EPG was smaller and that of sphingomyelin was larger in the white matter and cerebral cortex in Krabbe's disease—the difference from the normal cerebral cortex being more pronounced in the white matter These changes in Krabbe's disease might be due to the replacement of normal brain cells by astrocytes and globoid cells with a lipid pattern differing from that of the normal neural tissue

No previous study of the fatty acid patterns of the phosphoglycerides in Krabbe's disease is known to us The largest changes occurred in the white matter when compared with an appropriate control material The fatty acid patterns were quite similar to those found in the cerebral white matter of approximately 2-month-old infants Besides the fatty acid patterns showed a significantly higher concentration of arachidonic acid 20 4 (n-6) and an abnormally small 22 4 (n-6) The increased concentration of 20 4 (n-6) was also found in the cerebral cortex which indicates that this fatty acid might be enriched in the mesenchymal cells

The present study suggests that the main lipid changes of white matter in Krabbe's disease are the result of two events

(i) myelin loss which leads to an extensive reduction of cerebrosides sulfatides and cholesterol and a strong diminution of the monounsaturated fatty acids of the phosphoglycerides

(ii) replacement of neural tissue by cells of mesenchymal origin, which leads to a diminution of ethanolamine phosphoglycerides an increase of sphingomyelins and changed pattern of the polyunsaturated fatty acids.

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Fig 5 Fatty acid composition of major phosphoglycerides in cerebral white matter of normal infants and patients with Krabbe s disease

	EPG			SPG			CPG		
	Normals 6→27 months	Krabbe s disease 8–32 months (n=11)		Normals 6→27 months	Krabbe s disease 8–3 months (n=11)		Normals 6→27 months	Krabbe s disease 8–32 months (n=11)	
		Mean	S D		Mean	S D		Mean	S.D
16 0	6→4	7 7	2 0	5→2	5 4	1.5	44→3	46.8	2.3
16 1	0–1	1 0	0.3	0–1	0 7	0 3	2–4	3 9	0 9
18 0	16→9	20 7	1 9	44→45	37 6	2 0	14→16	8 4	1.3
18 1	23→33	17 0	3 9	23→39	24.5	3 6	32→42	31.3	2.0
20 1 (n 9)	4→6	1 1	0 4	2–3	2.4	0 7	0–1	0 6	0 1
20 3 (n-6)	1–3	1 7	0 4	1–2	2 1	0 6	0–1	0.9	0 2
20 4 (n-6)	14→11	20 4	1 6	5→2	5 7	0 7	5→3	5 1	1 1
22 4 (n-6)	19→22	9 6	2 1	6→3	4 4	1 0	0–1	0 8	0
22 5 (n-6)	4→2	4 2	1 4	3→1	4 6	2 3	0–1	0.4	0
22 6 (n 3)	8→7	14 9	3 6	6→3	11 3	3 7	0–1	1 1	0 3
Linoleic acid series	41→38	36 9	4.5	16→8	14 1	6 7	5–5	7 7	1 6
Linolenic acid series	9→8	14 9	3 6	5→	10.8	4 9	0–1	1 2	0.3

Values are weight percentages of methyl esters The following were detected in small amounts (in general less than 1%) and were added to the other values to make a total of 100% 18 (n-6) 18 3 (n-6), 20 3 (n-9) 22 5 (n 3) 4 4 (n-6)

fractions a decrease in the monoenoic acids and an increase in fatty acids of the linolenic acid series Within the linoleic acid family the ratio 20 4 (*n*-6)/22 4 (*n*-6) was more than one in both SPG and EPG which in normal brains occurs only in the grey matter after myelination has begun Of the saturated acids the concentration of 16 0 was high in all fractions while that of 18 0 varied it was diminished in SPG and in CPG while it was increased in EPG The results found for IPG differed only slightly from those in the control group

Myelin

The yield of myelin was very low in the 2 cases of Krabbe s disease only approx 2% of the yield from the normal 12 month-old infant The lipid patterns of the myelin from the brains of Krabbe s patients were similar to those of myelin isolated from the two normal brains Ethanolamine plasmalogens constituted the same portion of the ethanolamine phosphoglycerides as in normal myelin and had also the same dimethylacetal pattern The ratio phospholipids/cholesterol/galactolipids was 1 00 0 99 0 40 compared to 1 00 1 12 0 45 in the normal brains The cerebroside/sulfatide ratio was 3 0 1 in the myelin of Krabbe s disease and 4 0 1 in the myelin of normal brains

DISCUSSION

Hallervorden (12) was the first to point out the morphological similarities between Gaucher and globoid cells and to suggest that the globoid cells contained glycolipid-like material Brante (6) demonstrated a very lipid poor cerebral white matter and spinal cord in Krabbe s disease the concentrations of total lipids phospholipids and cholesterol were lower in the white matter than in cerebral cortex and the glycolipids of white matter were strongly reduced but Blackwood & Cummings (5) found an abnormally high glycolipid concentration in the white matter in one case

These early studies were performed on formalin-fixed material or on less typical cases of Krabbe s disease In 1961 Svennerholm

Acta Paediatr Scand 63 501–506 1974

CHEMICAL PATHOLOGY OF KRABBE'S DISEASE

II. *Fatty Acid Composition of Cerebrosides Sulfatides and Sphingomyelins in Brain*

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ABSTRACT Vanier M.-Th. and Svennerholm, L. (Department of Neurochemistry Psychiatric Research Centre University of Göteborg, Göteborg, Sweden). Chemical Pathology of Krabbe's Disease. II. Fatty acid composition of cerebrosides, sulfatides and sphingomyelins in brain. Acta Paediatr Scand, 63: 501 1974.—The fatty acid composition of sphingomyelins, cerebrosides and sulfatides was determined in cerebral tissue from 18 Swedish children aged 7–32 months, with Krabbe's disease. Sphingomyelins of the white matter had a fatty acid pattern which indicated a serious deficiency of myelin. The fatty acid pattern also suggested that a substantial amount of the sphingomyelins was derived from cells of mesenchymal origin. The cerebrosides, and to a certain extent the sulfatides of cerebral cortex and white matter had a higher concentration of saturated very-long-chain C_{22}–C_{26} acids than normal brains. The difference from the normal fatty acid pattern of cerebrosides was smaller in myelin. This suggests that the cerebrosides stored in the globoid cells have an abnormal fatty acid composition.

KEY WORDS: Myelin deficiency increase of saturated very-long-chain fatty acids

In an accompanying paper (12) we suggested that the lipid biochemical changes in brains from subjects who had died of Krabbe's disease were the combined effect of the storage of cerebrosides (galactosylceramides) in certain cells of mesenchymal origin, and a serious deficiency of myelin. The fatty acid patterns of the phosphoglycerides supported this concept. It was therefore important to know the fatty acid compositions of the sphingolipids which are particularly affected in this disease. Some results have been reported by one of us (4 7) by Menkes et al (3) and Eto & Suzuki (2). A further investigation was necessary however because the small number of investigated cases and the partly conflicting results did not allow conclusive interpretations.

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MATERIAL AND METHODS

Brain material

The material consisted of autopsy specimens of the brain from 18 cases of Krabbe's disease. The subjects had died at 7–3 months of age. A description of the brain material and its lipid composition has been given in the accompanying report (1). Myelin was prepared from cases (17). The control material consisted of 14 normal brains, aged 0–5 months (10).

Methods

Isolation of the sphingolipids

Individual samples and myelin Crude cerebroside sulfatide and sphingomyelin fractions were obtained by column chromatography on silicic acid after mild alkaline hydrolysis (1). Final separation and purification was achieved by thin-layer chromatography (11).

Large ale preparation Because of the low concentrations of the glycosphingolipids of cerebral cortex and sulfatides of white matter the material available from single brain was not enough for a reliable determination. A large scale preparation was, therefore undertaken. White matter (140 g) and cerebral cortex (250 g) were dissected and pooled separately from 6 brains of the subjects with Krabbe's disease. A 30-fold scaling-up of the column chromatography procedure de-

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Table 2. Composition of normal fatty acids in cerebrosides and sulfatides of cerebral white matter in Krabbe s disease

	Controls		Krabbe s disease			
	Cerebrosides	Sulfatides	Cerebrosides			Sulfatides
			Individuals (n = 16) 7–32 months		Large scale preparation 7–32 months	Large scale preparation 7–32 months
	0→25 months	0→25 months	Mean	S D		
Fatty acid						
16 0*	→1	4→1	1 9	0.7	1	2
18 0	17→5	23→	11.2	3	11	9
20 0	2→1	3→1	1 7	0 5	2	1
22 0	8→3	7→3	6.4	1.3	6	5
23 0	2→4	1→3	5.3	1.6	6	6
24 0	27→20	22→21	38 0	6 0	4	29
24 1	4→40	36→42	15.3	4 7	19	25
25 0	2→5	→4	4 6	1 4	3	4
25 1	2→6	1→6	2.1	1 1	1	3
26 0	2–	3→	4 3	0 8	3	4
26 1	8→12	7→14	7 9	2.0	6	12
16–22 saturated acids	29→10	37→7	1	3 8	20	17
23–26 saturated acids	34→30	37→29	52.2	7 0	54	43
16–26 mono-enoic acids	37→60	36→64	27 0	7.8	25	40

* Weight percentage of methyl esters.

cerebral cortex In Krabbe s disease as in normal brains very long-chain acids C_{23}–C_{26} acids predominated However their individual distribution was different from that normally seen in the age interval 0–25 months The saturated fatty acids C_{23}–C_{26}, particularly 24 0 and 24h 0 and to a lesser extent 23 0 and 23h 0 were increased while the monoenoic acids particularly 24 1 and 24h 1 were appreciably reduced A characteristic feature in Krabbe s disease was the very high ratio of 24 0/24 1 2 4 compared with 0.9 in normal newborn brains and 0.5 in 2 year-old normal brains In 3 cases K.R. 62, A.J 70 and A H 61 the ratio 24 0/24 1 was only 1 2–1 3 No correlation could be made with other biochemical parameters in these patients except for in case A.H 61 who had less pronounced lipid changes in brain than all the other cases (12)

The fatty acid patterns of the cerebrosides were analysed in the myelins of P.J 69 and A.J 70 and compared with those in the corresponding white matter In both cases the concentrations of 24 0 and 24h 0 were 6–9% lower with a corresponding increase of the monoenoic acids, 24 1 and 24h 1 but also of 26 1 and 26h 1

Sulfatides In the large scale preparation 2 hydroxy acids constituted 33% of total sulfatide fatty acids in white matter and 49% in cerebral cortex Very-long-chain C_{23}–C_{26} acids predominated.

The differences in fatty acid pattern from normal were less pronounced than in the cerebrosides Monoenoic acids had a concentration which was between that in normal newborns and 2 year-old infants The concentration of the C_{23}–C_{26} saturated acids was higher than that normally found in this age interval but the 24 0/24 1 ratio was only slightly higher than in the brains from newborns In the myelin preparations the fatty acid patterns were similar to those in the corresponding white matter

A difference between the fatty acid patterns of cerebrosides and sulfatides of white matter was thus found in Krabbe s disease

Table 1 *Fatty acid composition of sphingomyelins in cerebrum of patients with Krabbe s disease and of normal age matched infants*

	Cerebral cortex				Cerebral white matter					Myelin
	Controls (n=9) 0–25 months		Krabbe s disease (n=9) 7–32 months		Controls			Krabbe s disease (n=12) 7–32 months		Krabbe s disease (n=2)
	Mean	S D	Mean	S D	0	6	25 months	Mean	S D	13+25 months
Fatty acid										
16 0*	4 7	1 4	7 3	1 8	6	5	3	8 9	1 9	7
18 0	87 6	2 1	80 4	2 6	82	57	33	66 9	6 9	46
20 0	1 6	0.5	2 1	0 4	2	2	2	1 9	0 3	1
22 0	0 9	0 3	1 6	0 4	1	3	3	2.2	0 4	2
23 0	0 4	0.5	1 0	0 4	1	1	3	2 0	0.8	3
24 0	1 0	0 4	2.3	0 7	2	5	11	3 4	1.3	8
24 1	6	0.8	4 0	0 7	4	22	33	10.8	3 0	19
>24	0 6	0 1	0 8	0 4	1	4	11	2.4	1 7	8
Mono-enoic acids	3 7	1 3	5 6	0 8	5	26	42	13.5	3.8	25

*Weight percentage of methyl esters

scribed for small samples was used (1) Sulfatides were separated from the cerebrosides by column chromatography on DEAE-cellulose (9).

Fatty acid analyses

Acid methanolysis of the lipids separation and analysis of the fatty acid methyl esters by gas–liquid chromatography were performed as previously described (11) The fatty acid composition is reported as percentage of peak area (weight percentage).

RESULTS

No age changes in the fatty acid pattern of sphingomyelins cerebrosides or sulfatides were found in the brains of children with Krabbe s disease The mean value and standard deviation were therefore calculated In normal brains large variations of the fatty acid patterns occur with maturation (4 8 11) except for sphingomyelin fatty acids in cerebral cortex for which reason no mean value could be calculated The values of the control group are those for the youngest and oldest child

Sphingomyelins (Table 1)

In the cerebral cortex in Krabbe s disease 18 0 was the dominating fatty acid as in normal brains but its concentration was significantly lower while the concentration of 16 0 was higher Monoenoic acids and 24 0 were also increased

In the sphingomyelins of cerebral white matter the concentration of 18 0 was still very high compared with age matched controls and lay midway between those found in newborns and in a 6-month-old infant The concentration of 16 0 was abnormally high

In the myelin of 2 cases the concentration of 18 0 was considerably lower than in the corresponding white matter and a concomitant increase in the very-long-chain acids had occurred

Cerebrosides and sulfatides (Tables 2 and 3)

Cerebrosides and sulfatides were isolated and analysed in cerebral cortex and white matter of the large scale preparation The values found for the cerebrosides and sulfatides of cerebral cortex are not included in the tables because these lipids had identical fatty acid patterns in grey and white matter

Cerebrosides The values found in the large scale preparation were the same as those obtained in the individual samples The 2 hydroxy fatty acids constituted 52% of the total acids in white matter and 56% in the

that the degree of unsaturation was very low 24:1 being not more than 8–15% while 24:0 was correspondingly increased. This reversed ratio of 24:0/24:1 was also the most striking finding in studies from other laboratories (2, 3). We have found that not only 24:0 and 24h:0 had an abnormally high concentration but also the other very long-chain saturated acids particularly 23:0. The low degree of unsaturation was not restricted to 24:1 and 24h:1 but affected all the monoenoic acids. The high ratio of very-long-chain saturated C_{22}-C_{26} acids/monoenoic acids is a characteristic feature of the galactosylceramides of grey and white matter in Krabbe's disease and has not been found in other diseases studied in this laboratory. In the control brains this ratio is approximately 1:1 at birth and decreases with maturation to 0.5 at 2 years of age.

The fatty acid patterns of cerebrosides and sulfatides in brains of a given age are normally very similar (8, 11) with the exception of a higher content of hydroxy acids of cerebrosides than of sulfatides. The content of hydroxy acids of cerebrosides and sulfatides in Krabbe's disease was found to be within the normal range in the present study. The lower content of hydroxy acids reported by Menkes et al. (3) seems to be caused by contamination (11). The fatty acid patterns of cerebrosides and sulfatides in the present study differed. The sulfatides had a fatty acid composition which was more similar to the normal than the cerebrosides. The ratio of long-chain saturated acids/monoenoic acids was only slightly higher than in newborn brains and close to the values found in other myelin disorders. Eto & Suzuki (2) found that the sulfatides of white matter in their case had an essentially normal fatty acid composition. Also the cerebrosides of myelin had a fatty acid composition which was more normal and quite similar to that of the sulfatides.

Taken together these results suggest that the cerebrosides accumulated in the globoid cells have a fatty acid pattern which is seriously abnormal and differs in several respects from that in normal brains, particularly by a much higher content of saturated very-long chain acids.

The storage of cerebrosides with this particular fatty acid profile can be explained by various mechanisms. Cerebrosides are synthesized in large excess in the biosynthetic pools with the available fatty acids but only cerebrosides with a certain fatty acid pattern are incorporated in the myelin sheath or the plasma membranes of the oligodendroglial cells. All other cerebrosides are immediately degraded in the lysosomes but in Krabbe's disease, because of lack of cerebroside-β-galactosidase, they are accumulated in the globoid cells instead. Particularly cerebrosides with saturated very long-chain acids are biosynthesized in excess. The other possibility is that cerebrosides with monoenoic acids are preferentially degraded. In all brains with Krabbe's disease a slight residual activity of cerebroside β-galactosidase has been found (5 and unpublished results of M. Th. Vanier, G. Håkansson and L. Svennerholm) and this enzymatic activity may suffice for degradation of substantial amounts of cerebrosides with monoenoic acids.

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Table 3 Composition of 2 hydroxy fatty acids in cerebrosides and sulfatides of cerebral white matter in Krabbe s disease

	Controls		Krabbe s disease			
	Cerebrosides	Sulfatides	Cerebrosides			Sulfatides
			Individuals (n = 16) 7–32 months		Large scale preparation 7–32 months	Large scale preparation 7–3 months
	0→25 months	0→25 months	Mean	S D		
Fatty acid						
18h 0[a]	1–1	4→1	1 3	0.5	1	1
20h 0	1–1	1–1	0 8	0 3	1	1
22h 0	11→9	13→8	8 8	1 3	10	9
23h 0	8→12	7→9	12.2	2.	11	8
24h 0	44→43	40→41	53 2	4 4	52	49
24h 1	16→18	19→22	7	2 6	9	14
25h 0	3→6	4→5	5.5	1 3	6	5
25h 1	1→3	1→3	0 8	0 5	1	1
26h 0	4–	3–3	3 9	0 7	4	5
26h 1	8–7	6–8	4 6	1 4	4	7
16–23 saturated acids	21→10	27→9	10 9	1 8	12	11
23–26 saturated acids	61–62	51→58	74	3 3	73	67
16–26 mono-enoic acids	18→28	[illegible]→3	14 4	3 7	15	23

[a] Weight percentage of methyl esters

This finding was verified in 5 individual cases in which the sulfatides could also be analysed 24 0 was lower 24 1 and 26 1 higher than in the corresponding cerebrosides while the *other fatty acids did not vary*

DISCUSSION

Sphingomyelins

Before myelination 18 0 is the predominating fatty acid of sphingomyelins in both grey and white cerebral matter In cerebral cortex the pattern remains unchanged while in the white matter the fatty acid composition shows an extensive and early change with maturation (4 11) The concentration of 18 0 falls rapidly while the proportion of the very long chain acids increases at a corresponding rate In a previous report from this laboratory a much lower concentration of very long-chain fatty acids was found in cases of Krabbe s disease compared with that in age matched controls (4) In the present study *the concentration of the* C_{22}–C_{26} *fatty acids of* cerebral white matter in Krabbe s disease was the same as in the 1–2-month-old normal infant brains (11) These changes could be considered to reflect the myelin deficiency in Krabbe s disease *Some other features in the* fatty acid pattern—the increased concentration of 16 0 in white matter and cerebral cortex and the increased concentration of 22 0 24 0 and 24 1 in cerebral cortex—suggest some origin other than the ectodermal nervous tissue It has previously been shown (6) that extraneural organs with a large number of mesenchymal cells have a high concentration of 24 0 and 24 1 and differ from those of cerebral tissue by a higher concentration of 16 0 and 22 0 We suggest that the sphingomyelins with the above mentioned fatty acids in brains of patients with Krabbe s disease are mainly derived from the mesenchymal cells

Cerebrosides and sulfatides

In 1965 Svennerholm (7) reported the distribution of the normal fatty acids of the cerebrosides in Krabbe s disease He pointed out

that the degree of unsaturation was very low 24 1 being not more than 8–15 % while 24 0 was correspondingly increased This reversed ratio of 24 0/24 1 was also the most striking finding in studies from other laboratories (2 3) We have found that not only 24 0 and 26 0 had an abnormally high concentration but also the other very-long-chain saturated acids, particularly 23 0. The low degree of unsaturation was not restricted to 24 1 and 26 1 but affected all the monoenoic acids The high ratio of very long-chain saturated C_{23}–C_{26} acids/monoenoic acids is a characteristic feature of the galactosylceramides of grey and white matter in Krabbe's disease, and has not been found in other diseases studied in this laboratory In the control brains, this ratio is approximately 1 1 at birth, and decreases with maturation to 0.5 at 2 years of age

The fatty acid patterns of cerebrosides and sulfatides in brains of a given age are normally very similar (8 11) with the exception of a higher content of hydroxy acids of cerebrosides than of sulfatides The content of hydroxy acids of cerebrosides and sulfatides in Krabbe's disease was found to be within the normal range in the present study The lower content of hydroxy acids reported by Menkes et al (3) seems to be caused by contamination (11) The fatty acid patterns of cerebrosides and sulfatides in the present study differed. The sulfatides had a fatty acid composition which was more similar to the normal than the cerebrosides The ratio of long-chain saturated acids/monoenoic acids was only slightly higher than in newborn brains and close to the values found in other myelin disorders Eto & Suzuki (2) found that the sulfatides of white matter in their case had an essentially normal fatty acid composition. Also the cerebrosides of myelin had a fatty acid composition which was more normal and quite similar to that of the sulfatides

Taken together these results suggest that the cerebrosides accumulated in the globoid cells have a fatty acid pattern which is seriously abnormal and differs in several respects from that in normal brains particularly by a much higher content of saturated very-long chain acids

The storage of cerebrosides with this particular fatty acid profile can be explained by various mechanisms Cerebrosides are synthesized in large excess in the biosynthetic pools with the available fatty acids but only cerebrosides with a certain fatty acid pattern are incorporated in the myelin sheath or the plasma membranes of the oligodendroglial cells All other cerebrosides are immediately degraded in the lysosomes but in Krabbe's disease because of lack of cerebroside β-galactosidase they are accumulated in the globoid cells instead. Particularly cerebrosides with saturated very long-chain acids are biosynthesized in excess The other possibility is that cerebrosides with monoenoic acids are preferentially degraded In all brains with Krabbe's disease a slight residual activity of cerebroside β-galactosidase has been found (5 and unpublished results of M Th Vanier G Håkansson and L Svennerholm) and this enzymatic activity may suffice for degradation of substantial amounts of cerebrosides with monoenoic acids

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PLASMA AND BLOOD VOLUMES IN SEVERELY MALNOURISHED INFANTS

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ABSTRACT Zoumboulakis, D., Anagnostakis, D., Kiossoglou, K., Agathopoulos, A. and Tsenghi, C. (Department of Paediatrics, Athens University Athens, Greece). Plasma and blood volumes in severely malnourished infants. Acta Paediatr Scand, 63: 507 1974. —This study was carried out in order to gain some insight into the plasma and blood volume fluctuations in severely malnourished infants. By means of an isotope dilution technique and microhaematocrit measurements, plasma and blood volumes were studied in 18 severely malnourished infants, aged 5 to 20 months and in 5 healthy controls, aged 3 to 20 months. Both plasma and blood volumes were found to be considerably higher in the malnourished infants (79.2±14.5 ml/kg and 119±17.0 ml/kg, respectively) than those in the controls (51.6±6.8 and 76.4±7 7 mg/kg respectively The differences in both instances were statistically highly significant. When, however plasma and blood volumes were expressed not by means of the actual body weight but per kg of the expected body weight for height, there were no significant differences between malnourished (50.6±9 4 ml/kg and 76 1±12.4 ml/kg respectively), and healthy infants (51.6±6.8 ml/kg and 76.4±7 7 ml/kg respectively) These results suggest that the absolute intravascular fluid volume, which plays an important role in maintaining normal homeostasis, is unchanged in malnutrition.

KEY WORDS: Malnutrition, blood volume plasma volume

The expansion of extracellular fluid is a well documented feature of malnutrition in adults (1 2) and children (3 4) In addition, several investigators have reported an increased plasma and blood volume in infantile malnutrition but the results obtained differ widely (5 6 7) Since these discrepancies can be only partially attributed to the different methods used it is interesting to determine whether other factors may be involved

The reported reduction of glomerular filtration rate and renal plasma flow in malnutrition (8 9) gives rise to the question of whether renal disorders may be the result of a reduction in circulating plasma or blood volume

The present study was carried out in the hope of gaining some insight into the plasma and blood volume fluctuations which may play a role in the pathogenesis of severe malnutrition

MATERIAL AND METHODS

Eighteen infants (11 male and 7 female) aged 5 to 20 months, with severe malnutrition, were studied as soon as possible after their admission to the hospital and before any therapy was instituted. All were under weight for their age mean weight 64 7% of the expected weight for height, according to the anthropometric charts and tables, issued by the Harvard School of Public Health (10). They were hypoproteinaemic and 6 of them were oedematous. Almost all of these infants belonged to a population of gypsies of poor economic status their clinical history revealed a very poor protein intake.

Treatment consisted essentially of graduated feeds,

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Table 2. *Clinical data in malnourished infants*

Case no.	Sex	Age (mo)	Present body weight (kg)	Height (cm)	Expected body weight (kg)	Present WB as % of the expected	Haematocrit %
1	M	5	4.0	65	6 8	59	38
	M	6	4.5	66	7.6	59	25
3	F	7	4 0	68	7.8	51.5	40
4	M	8	5 8	69	8 4	69.5	38
5	M	9	4 0	69	9 0	44 5	25
6	F	9	3 9	70	9.0	43	43
7	M	1	6 6	75	10	66	40 5
8	M	13	6.1	76	10.2	60	40 5
9	F	13	7 3	76	10.	71.5	42 5
10	M	14	7.8	77	10 4	75	40.5
11	F	14	6.3	77	10.4	60.5	42.0
12	F	14	7 7	76	10 4	74	38.5
13	F	15	8.2	79	10 6	77.5	34 5
14	M	15	6.9	78	10 7	65	48.0
15	M	15	6 9	79	10 9	63.5	35.5
16	M	15	8 5	78	10 7	80	39.5
17	M	17	8 3	81	11	74	36 0
18	F	20	8 7	85	1 0	7? 3	43 0

Table 3 *Blood and plasma volume in malnourished infants*

Case no	Plasma volume (ml)	Plasma volume/ present BW (ml/kg)	Plasma volume/ expected BW (ml/kg)	Blood volume (ml)	Blood volume/ present BW (ml/kg)	Blood volume/ expected BW (ml/kg)
1	410	102	60	612	156	90
	504	112	66	640	142	84
3	346	86	44	535	134	68
4	365	62	44	547	94	65
5	339	85	38	433	108	48
6	328	84	37	525	134	98
Mean ± S D for infants <12 months		88 5±17 2			128±22.9	
7	905	76	90	780	118	78
8	906	83	49	781	128	76
9	406	69	49	805	110	79
10	538	69	52	830	106	80
11	570	83	50	820	130	79
12	547	71	53	825	107	79
13	788	96	74	1 113	138	106
14	449	65	42	775	11	73
15	603	87	55	875	177	80
16	540	63	51	825	97	78
17	6.5	75	56	910	110	81
18	908	99	42	815	94	68
Mean ± S D for infants >12 months		74 6±10 9			114±13.6	
Mean ± S D for all infants	496 ±117	79 2 ±14 5	50 6 ±9 4	747 ±159	119 1 ±17 8	76 1 ±1_4

Table 1 *Clinical data and plasma and blood volume values in the control group*

Case no	Sex	Age (mo)	Present body weight (kg)	Height (cm)	Plasma volume (ml)	Plasma volume/BW (ml/kg)	Blood volume (ml)	Blood volume/BW (ml/kg)	Haematocrit %
1	♀	3	5.7	60	361	63	506	89	33
2	♂	8	8.4	69	467	53	656	75	33
3	♂	9	8.0	71	414	46	612	68	37
4	♂	20	12.0	85	569	48	890	74	41.5
5	♀	20	1[illegible].0	86	578	48	915	76	42
Mean						51.6		76.4	
±S D						6.8		7.7	

therapy of any associated infection and administration of ferrous sulphate folic acid and potassium supplements

Five healthy children aged 3 to 20 months served as controls Tables 1 and 2 summarize data obtained from the infants studied

Plasma volume was determined by isotope dilution following intravenous injection of 3 μCi of ^{131}I labelled human albumin Plasma radioactivity was measured in an electronic counter (Volemetron Atomium Corporation) on a single blood sample obtained 10 minutes after the injection Haematocrit was measured in a micro-haematocrit (Adams Autocrit Centrifuge) From the haematocrit value (Hct) obtained the corrected venous haematocrit (CVH) was calculated as follows (11)

$$CVH = Hct \times 0.96 \text{ and}$$

the whole blood haematocrit (WBH) was estimated thus (12)

$$WBH = CVH \times 0.91$$

The blood volume was then calculated as follows

$$\text{blood volume} = \frac{\text{plasma volume}}{1 - WBH}$$

Plasma and blood volumes were not measured on recovery

RESULTS

The plasma volume expressed as ml of plasma per kg of actual body weight was considerably higher in all malnourished infants (79.2±14.5 ml/kg) than in the controls (51.6±6.8 ml/kg) as seen in Tables 1 and 3 The difference is statistically highly significant ($p<0.001$)

In addition the blood volume (ml/kg of actual body weight) in all malnourished infants was higher than in the healthy ones (119±17.8 versus 76.4±7.7 ml/kg) The difference is statistically highly significant ($p<0.001$) When however the plasma and blood volumes were expressed not by means of the actual body weight but per kg of the expected weight for height there were no significant differences between malnourished and healthy infants The mean plasma volume of malnourished infants was 50.6±9.4 ml/kg of expected weight versus 51.6±6.8 ml/kg in controls and the blood volumes were 76.1±12.4 ml/kg of expected weight versus 76.4±7.7 ml/kg respectively (Tables 1 and 3)

When the age of the malnourished infants is taken into consideration there is a difference between those under and those above 1 year of age (Table 3) in infants less than 12 months the plasma volume was 88.5±17.2 and the blood volume 128±22.9 ml/kg whereas the corresponding values for infants over one year were 74.6±10.9 and 114.7±13.6 ml/kg The difference in plasma volume between infants below and above 1 year is statistically probably significant ($p=0.05$) On the other hand no statistical difference was found between the two age groups in blood volume ($p>0.1$)

The relationship between plasma and blood volume expressed as ml/kg of actual body weight against weight deficit is shown in Fig 1 There is a linear correlation between these parameters the greater the weight de

The assumption that the degree of malnutrition is a determining factor for the relative plasma and/or blood volume is further supported by the fact that Gomez et al (7) studying a population with a high degree of malnutrition reported values for plasma and blood volumes similar to ours (83 0 and 135 0 ml/kg respectively) Their values for plasma and blood volumes in children recovered from malnutrition were higher than those of our healthy infants This may be attributed to the different method used (Evans blue) or to the different conditions of the groups studied

Another possible reason for the discrepancies reported in the values of plasma and blood volumes is the difference in age groups of the infants studied. In fact our study suggests that there may be a difference in plasma and blood volume for infants below and above one year of age this finding is not mentioned by other investigators

As far as haematocrit is concerned if one groups the mean values for infants below and for those above one year of age these are almost identical in controls and malnourished infants for both groups.

When the plasma and blood volumes of malnourished infants are expressed in terms of expected weight for height they were identical with those for healthy infants This indicates that the absolute intravascular fluid volume was unchanged This is in good agreement with the assumption that it is the absolute intravascular fluid volume rather than its size to the body weight which is important in maintaining normal homeostasis in malnutrition (4)

It is logical to assume therefore that the changes in renal function which occur in malnutrition (8 9) are not due to reduction in circulating plasma or blood volume These alterations are probably part of the necessary mechanisms by which the malnourished organism conserves the absolute fluid volume of the intravascular space and represent an effort in adaptation phenomenon which permit survival of the malnourished children

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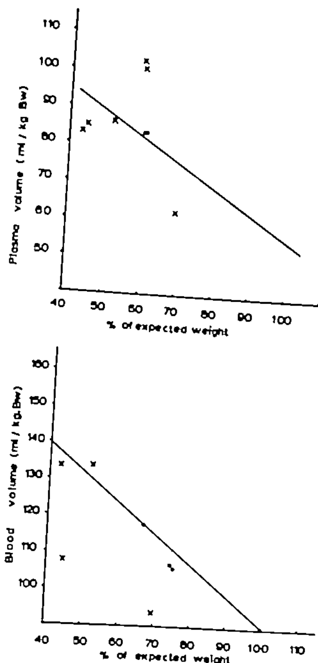


Fig 1 Plasma (above) and blood (below) volumes in malnourished infants related to their percentage of expected weight for height For plasma $y=-0.65$ $x=+121$ $r=-0.47$ and $p<0\ 05$ For blood $y=-0\ 805$ $x=+171$ $r=-0\ 47?$ and $p<0\ 05$ Symbol x stands for infants <12 months and symbol ● stands for infants >12 months.

ficit the higher the values of plasma and blood volume

The mean haematocrit values for patients and controls below 12 months are 35 6% and 34 5% respectively and for those above 12 months of age 40 0% and 41 7% respectively

DISCUSSION

The method used in this study for the estimation of plasma and blood volumes although not ideal is reliable enough for all practical purposes (5 6) Although a leakage of the injected isotope at a capillary level may affect the plasma volume this would be a remote possibility

The expansion of the extracellular fluid is a constant feature of infantile malnutrition, and increased plasma volume has been reported irrespective of the methods used (4 5 6 7) The results reported however differ widely Alleyne (5) using the same method as us found in normal infants a mean value for plasma volume of 49 3 ml/kg and for blood of 72 6 ml/kg Cohen & Hansen (6) found a mean value for plasma volume of 51 0 ml/kg These values are all strikingly close to those obtained in the present study for the control group

The values however for plasma and blood volumes in malnourished infants obtained in our study (79 2±14 5 and 119±17 8 respectively) are considerably higher than those reported by other investigators Plasma and blood volumes in malnourished infants were respectively 62 9 and 80 4 ml/kg in Alleyne s study (5) whereas in another study (13) the plasma volume in malnourished infants was 60 0 ml/kg

The differences between our values and those of Alleyne for plasma and blood volumes in malnourished infants are difficult to explain One explanation could be the difference in the degree of malnutrition Since the relative plasma and blood volume is in direct relationship to the weight deficit it is evident that the greater the weight wastage the higher the value of plasma and blood volumes expressed as ml/kg In the present study the mean weight wastage of the malnourished infants was 35 3% of the expected weight for height in contrast to a weight wastage of only 25 7% in the study of Alleyne (5) although the age distribution in both studies was almost identical

The assumption that the degree of malnutrition is a determining factor for the relative plasma and/or blood volume is further supported by the fact that Gomez et al (7) studying a population with a high degree of malnutrition reported values for plasma and blood volumes similar to ours (83 0 and 135 0 ml/kg respectively) Their values for plasma and blood volumes in children recovered from malnutrition were higher than those of our healthy infants This may be attributed to the different method used (Evans blue) or to the different conditions of the groups studied

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ENURESIS

An Attempt at Classification by Genesis

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ABSTRACT Andersen O Ortved and Petersen K E. (Children's Hospital Fuglebakken Copenhagen Denmark) Enuresis An attempt at classification by genesis Acta Paediatr Scand 63 512 1974.—A series of 133 enuretic children aged 4–15 years is reported Age variation sex ratio and distribution of anamnestic data substantiate that the sign enuresis comprises an extremely heterogeneous group The disease is therefore probably due to several causes. In an effort to elucidate in greater detail the causal relationship int al with a view to its therapeutic significance the material was divided into 4 groups (A) Children with primary enuresis without behavioural disturbances. (B) Children with primary enuresis and behavioural disturbances. (C) Children with secondary enuresis without behavioural disturbances. (D) Children with secondary enuresis and behavioural disturbances. It is demonstrated that boys, especially young boys, aged 4–6 years usually belong to group A whereas girls, in particular those aged 7–10 years, predominate in groups B+C+D In girls the enuresis was more often of a diurnal nature either in the form of isolated diurnal or combined diurnal and nocturnal enuresis, especially in girls of groups B+C+D Symptoms of urinary tract infection were also more common in girls than in boys, and predominantly among the girls of groups B+C+D. Encopresis was twice as common in boys as in girls, also mainly in groups B+C+D Data concerning a familial predisposition and heavy sleep could not contribute to a further pathogenetic elucidation

KEY WORD: Enuresis

Numerous investigations and therapeutic attempts bear witness to the considerable discomfort caused by enuresis not only to the patients but also to their next-of kin Enuresis presumably has many causes With special reference to a more rational treatment it would be of great value to be able to classify the condition into groups of different genesis The present paper reports such an attempt based upon an analysis of enuretic children admitted to a children's hospital

MATERIAL AND METHOD

During a period of 14 months, 142 children were admitted with a diagnosis of enuresis to the Children's Hospital, Fuglebakken Copenhagen Primarily 7 were excluded from the material as they were under 4 years of age So were 2 children in whom it was not possible to rule out malformations of the urinary tract as a cause of the enuresis This left 133 children 75 boys and 58 girls in the age range 4–15 years

By standardized questions it was learnt whether there was a *family history of enuresis meaning the occurrence of enuresis* in one of the parents or siblings. It was asked whether the enuresis was primary i e had always existed or secondary defined as a state where the child had had a period exceeding 1 month without wetting On the basis of the anamnestic data obtained, we tried to evaluate urinary urgency and frequency sleep level and whether the children had had symptoms or signs that could be interpreted as urinary tract infection i e a combination of burning on urination abdominal pain and fever From the data thus collected we tried to obtain an overall evaluation of the child's psyche behavioural disturbances the nature of the milieu and the child's reaction to the enuresis Behavioural disturbances was considered when the parents regarded the behaviour of the child as deviating from

Table 1 *Classification of the material into groups*

The figures indicate the number of children, in parentheses the percentage distribution

Groups	Boys		Girls		Boys+Girls	
A Prim. enur. without behavioural disturbances		48 (64)		26 (45)		74 (55)
B Prim. enur. with behavioural disturbances	13		4		17	
C Sec. enur. without behavioural disturbances	6	27 (36)	22	32 (55)	28	59 (45)
D Sec. enur. with behavioural disturbances	8		6		14	
Total		75 (100)		58 (100)		133 (100)

their normal concepts, or when the authors considered environmental factors as emotional strains.

During the stay in hospital microscopic examination of the urine was done 3 times, the specific gravity of the urine was determined, and the urine was tested for glucose and protein. If necessary, X-ray examination of the kidneys was done, EEG, psychological testing, or further elucidation of social circumstances.

On the basis of the anamnestic data the children were divided into 4 groups:

Group A: Children with primary enuresis without behavioural disturbances

Group B: Children with primary enuresis and behavioural disturbances

Group C: Children with secondary enuresis without behavioural disturbances

Group D: Children with secondary enuresis and behavioural disturbances

RESULTS

Sex ratio

The material of 133 children consisted of 56% boys and 44% girls.

Grouping

Table 1 shows that group A was of the same size as groups B, C and D combined.

From the percentage distribution it is apparent that boys were predominantly classified in group A (64%), whereas the girls were more equally distributed on group A (55%) and groups B+C+D (45%).

Age distribution

At admission the boys were on the average 8 years old, the girls 7½ years. This difference is not significant (Wilcoxon test $p>0.1$). From Table 2 it may be seen that young boys, unlike young girls, had predominantly enuresis without behavioural disturbances (group A). Moreover, a marked representation of older boys is observed in this group.

Table 3 gives the percentage occurrence of various important characteristics and symptoms in the entire material, among boys and girls and in the 2 main groups.

Treatment

65% of the children had been treated at home by scheduled wakening at night. 35%

Table *Distribution by age and groups*

The figures in parentheses indicate the percentage distribution of the age groups among girls and boys

Group	Boys A	Boys B	Boys C	Boys D	Boys B–D	Boys A–D	Girls A	Girls B	Girls C	Girls D	Girls B–D	Girls A–D
4–6 years	23	2	4	3	9	32 (43)	12	0	8	1	9	21 (36)
7–10 years	19	8	1	5	14	33 (44)	13	4	13	5	22	35 (60)
11–15 years	6	3	1	0	4	10 (13)	1	0	1	0	1	2 (4)

Acta Pædiatr Scand 63 512–516 1974

ENURESIS

An Attempt at Classification by Genesis

OLE ORTVED ANDERSEN and KNUD F. PETERSEN

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ABSTRACT Andersen O Ortved and Petersen K F (Children's Hospital Fuglebakken Copenhagen, Denmark) Enuresis. An attempt at classification by genesis. Acta Paediatr Scand 63 512 1974.—A series of 133 enuretic children aged 4–15 years is reported Age variation sex ratio and distribution of anamnestic data substantiate that the sign enuresis comprises an extremely heterogeneous group The disease is therefore probably due to several causes. In an effort to elucidate in greater detail the causal relationship int al. with a view to its therapeutic significance the material was divided into 4 groups. (A) Children with primary enuresis without behavioural disturbances. (B) Children with primary enuresis and behavioural disturbances. (C) Children with secondary enuresis without behavioural disturbances. (D) Children with secondary enuresis and behavioural disturbances. It is demonstrated that boys, especially young boys aged 4–6 years, usually belong to group A whereas girls in particular those aged 7–10 years predominate in groups B+C+D In girls the enuresis was more often of a diurnal nature either in the form of isolated diurnal or combined diurnal and nocturnal enuresis, especially in girls of groups B+C+D Symptoms of urinary tract infection were also more common in girls than in boys and predominantly among the girls of groups B+C+D Encopresis was twice as common in boys as in girls, also mainly in groups B+C+D Data concerning a familial predisposition and heavy sleep could not contribute to a further pathogenetic elucidation

KEY WORD Enuresis

Numerous investigations and therapeutic attempts bear witness to the considerable discomfort caused by enuresis not only to the patients but also to their next-of kin Enuresis presumably has many causes With special reference to a more rational treatment it would be of great value to be able to classify the condition into groups of different genesis The present paper reports such an attempt based upon an analysis of enuretic children admitted to a children's hospital

MATERIAL AND METHOD

During a period of 14 months, 142 children were admitted with a diagnosis of enuresis to the Children's Hospital, Fuglebakken Copenhagen Primarily 7 were excluded from the material as they were under 4 years of age So were children in whom it was not possible to rule out malformations of the urinary tract as a cause of the enuresis This left 133 children 75 boys and 58 girls in the age range 4–15 years

By standardized question it was learnt whether there was a family history of enuresis meaning the occurrence of enuresis in one of the parents or siblings. It was asked whether the enuresis was primary i e had always existed or secondary defined as a state where the child had had a period exceeding 1 month without wetting On the basis of the anamnestic data obtained we tried to evaluate urinary urgency and frequency sleep level and whether the children had had symptoms or signs that could be interpreted as urinary tract infection i e a combination of burning on urination abdominal pain and fever From the data thus collected we tried to obtain an overall evaluation of the child's psyche behavioural disturbances, the nature of the milieu and the child's reaction to the enuresis Behavioural disturbances was considered when the parents regarded the behaviour of the child as deviating from

es. Like Øster (20) we found a somewhat lower frequency A uniform incidence in all groups indicates that the predisposition is not merely a genetic *but also an exogenous* factor

The enuresis was of a nocturnal nature in more than 90% of the children. Diurnal and nocturnal or isolated diurnal enuresis occurs mainly in girls (Table 3) Isolated diurnal enuresis was present in 5 out of 28 girls with secondary enuresis as compared with 1 out of 30 girls with primary enuresis and in 3 out of 75 boys. During the stay in hospital there occurred a change into isolated nocturnal enuresis in boys as well as girls, within the entire series from 52 to 64% indicating that isolated diurnal enuresis is a less resistant condition. The distribution of isolated nocturnal isolated diurnal and nocturnal plus diurnal enuresis was largely the same in our series as that found by others (2 4 8 16 20)

Martin (9) has reported that in a control series 20% of the children were characterized as heavy sleepers In an enuretic material he found a frequency of 63% Generally the frequency is stated to be somewhat higher viz. 70–80% (8 12, 16) We were told that 76% of the boys and 61% of the girls with enuresis were heavy sleepers The high frequency found by us as well as by others must be regarded with the reserve that the data are based upon a subjective estimate In this connection it would be of value to obtain objective proof for instance by an EEG investigation during sleep

In a very large material Dodge et al (3) found about 2% of all the girls to have bacteriuria as compared to 0 1% of the boys. About 6% of the girls with nocturnal enuresis had bacteriuria. Furthermore *these* authors could demonstrate a correlation between the degree of severity of enuresis and the frequency of bacteriuria. We also found that girls had signs of urinary tract infection more often than boys. Lake (8) and Shaffer et al (17) observed signs of urinary tract infection in 7% and 12% respectively of the children in their series

Urinary frequency and imperative urgency were ascertained by McKendry et al (11) with approximately the same frequency as in our material

In our experience encopresis occurred with the same frequency at home as in hospital indicating that this condition is fairly resistant to treatment In the material as a whole we found a somewhat higher frequency of encopresis than others (7 16 20) (15–20%) It should be noticed that this sign occurred twice as often in boys as in girls in particular in groups B+C+D

The sex difference age difference and different distribution of several signs in the groups highly suggest a different pathogenesis of the enuresis A number of authors have previously divided enuresis into a primary and a secondary type and Gellis (5) as well as Palmisano (13) have suggested a division into 3 groups

From a pathogenetic point of view we tried primarily to divide our patients into 3 groups by symptoms and signs that might indicate (1) Delayed development of bladder control These children were characterized mainly by having primary nocturnal enuresis by being heavy sleepers and showing no signs of major behavioural disturbances (2) Small bladder capacity characterized mainly by the children having urinary frequency imperative urgency and primary enuresis without major behavioural disturbances (3) Psychogenesis i e children with behavioural disturbances or exposed to emotionally stressing environmental factors often with secondary nocturnal and/or diurnal enuresis

However such a classification was rendered difficult by some overlapping of the groups and by the problems of assessing the anamnestic criteria. For instance maintaining this classification would have made a comparison with other materials difficult. Until there are possibilities of an objectively

Table 3 *Occurrence of various characteristics and symptoms*
The figures represent the frequency in per cent partly in the entire series and partly in the main groups

	Boys+Girls			Girls			Boys		
Group	A	B–D	A–D	A	B–D	A–D	A	B–D	A–D
Predisposition	42	37	40	50	31	40	38	40	40
Nocturnal enuresis	57	46	52	58	34	45	56	59	57
Nocturnal and diurnal enuresis	38	46	41	38	50	45	38	41	39
Diurnal enuresis	5	8	7	4	16	10	6	0	4
Heavy sleep	69	67	68	59	62	61	77	73	76
Urinary frequency	41	50	45	47	58	54	37	30	35
Imperative urgency	41	65	51	40	74	58	42	50	44
Presumed urinary tract infection	9	17	13	8	28	19	10	4	8
Encopresis	24	27	26	15	16	16	29	41	33

had received some kind of drug but only 4% reported a favourable effect thereof During the stay in hospital 29% of the children showed improvement defined as a decrease in the frequency of enuresis of 2 nights or more a week While in hospital 15 children were treated with drugs mainly ephedrine or dexedrine with a favourable effect in one third Fourteen were treated by a waking device leading to improvement in 2

1–2 years after discharge all the children were offered an out patient examination and if desired drug therapy (14) Apart from this no follow-up was carried out

DISCUSSION

Several authors have previously studied the occurrence of various characteristics and symptoms in series of enuretic patients selected on the basis of various criteria The discrepancy in the occurrence of the symptoms and signs seems to be due partly to a difference in the definition and partly to a difference in the composition of the materials

As there was a preponderance of boys in our material and as several symptoms and signs occurred with a different frequency in boys and girls we felt we had to divide the material by sex

68% of the children had primary enuresis (Table 1) but when distributed by sex this was found to concern about 80% of the boys and only about 50% of the girls Apart from Martin (9) who found this condition in 40% of a material of 4293 children the frequency in the present series was in agreement with that observed by others viz. 70–80% (4 11 15 20) Like Martin (9) we found that in older children the enuresis was most often primary (80%)

Emotional disturbances behavioural difficulties and environmental faults have been defined in different ways in the studies quoted below Moreover the materials on which the assessments are based are of different compositions which renders direct comparison difficult Øster (20) found that 72% of the children were from a predisposed environment Uhrbrand & Nobel (18) found environmental faults in 66% Keiser Nielsen (7) judged 50% as giving behavioural trouble Noack (12) characterized 90% as problem children and according to Werry & Cohtsen (19) 50% of the children in their material had emotional disturbances In contradistinction we found in the present series by an anamnestic assessment emotional disturbances in only 23% of the children and during the stay in hospital only 10% exhibited a deviant behaviour

Several authors (1 4 6 12 18) have reported that 50–80% of the children in their materials have had a predisposition to enure

us. Like Øster (20) we found a somewhat lower frequency A uniform incidence in all groups indicates that the predisposition is not merely a genetic but also an exogenous factor

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However such a classification was rendered difficult by some overlapping of the groups and by the problems of assessing the anamnestic criteria For instance maintaining this classification would have made a comparison with other materials difficult Until there are possibilities of an objectively

measurable assessment of the vague anamnestic criteria we feel it is reasonable to rest content with a classification into marked reproducible signs By our classification we have tried to set up two characteristic extremes group A primary enuresis without behavioural disturbances and group D secondary enuresis with behavioural disturbances in an attempt at extracting at least the group in which external factors appear to be of decisive pathogenetic significance Since environmental factors in particular are frequently factors which have persisted throughout childhood there is a risk that such a classification may erroneously characterize a secondary enuresis conditioned by external factors as being primary Moreover MacKeith (10) believes that fear provoking episodes about 3 years of age cannot be excluded as a cause of enuresis which in that case would be characterized as primary We therefore decided to treat group B (primary enuresis with behavioural disturbances) together with groups C and D realizing that thereby we were presumably including a few children belonging to group A who may thus have been erroneously classified as having behavioural disturbances

The investigation has clearly shown that further research is required in the heterogeneous group of enuresis and that the most urgent problem is an attempt at measuring objectively the patients motley symptom complex

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DEVELOPMENTAL STUDY OF THE RENAL RESPONSE TO AN ORAL SALT LOAD IN PRETERM INFANTS

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ABSTRACT Aperia, A., Broberger O., Thodenius, K. and Zetterström, R. (Department of Paediatrics, Karolinska Institutet, St Göran's Children Hospital Stockholm, Sweden). Developmental study of the renal response to an oral salt load in preterm infants. Acta Paediatr Scand 63 517 1974.—An evaluation of sodium homeostasis in 44 preterm infants with gestational ages between 29 and 37 weeks has been carried out during the first week after birth and until time of expected term. The natriuretic response to an oral sodium load has been studied in all infants and the GFR (single injection technique of inulin) in 17 infants. The results are compared with those previously found in full-term infants. The natriuretic response was highest and the GFR was lowest in the very preterm neonates. In the very preterm infants the values for sodium excretion and GFR was just about the same at the time of expected term as in full-term newborns. Various explanations for the difference between the very preterm neonates and full-term neonates are discussed. One factor of importance might be the anatomical development. The immature kidney has in comparison to the adult kidney relatively larger glomerular than tubular mass. Extra-uterine life seems to have little influence on the development of GFR as well as on the development of the response to the oral salt load. Thus in the very preterm infants, the postmenstrual rather than the postnatal age should be considered when prescribing fluid, electrolytes and drugs.

KEY WORDS: Newborn, preterm renal function glomerular filtration rate, sodium excretion, water diuresis

The renal response to an oral sodium load has previously been reported to be low in newborn full-term infants (3) In preterm infants glomerular filtration rate (GFR) is lower than in full-term infants and older children (4 36). One might therefore speculate that the ability to excrete sodium is even lower in preterm infants than in infants born at term

In the present report the renal response to an oral salt load has been studied in newborn preterm infants of various gestational ages The results have been compared with those previously found in full-term infants (3) and in older children (15) The postnatal development of the renal sodium elimination and glomerular filtration rate (GFR) has been followed until 40 weeks of postmenstrual age

MATERIAL AND METHODS

Forty-nine studies have been performed in 44 healthy preterm infants of gestational ages varying between 29 and 37 weeks. One infant was studied 3 times and 3 were studied twice. In addition, 3 full-term infants have been investigated. Gestational age was calculated from the first day of the mother's last menstrual period until the day of birth. In order to confirm the gestational

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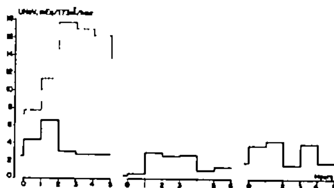


Fig 1 Hourly natriuretic response to an oral salt load of 0 12 g/kg body weight in three different preterm infants. All studies during first week after birth natriuretic response to the same load in a representative study in the older children

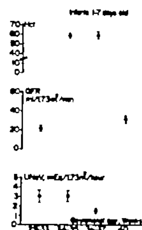


Fig 2 Mean values of average hourly urine sodium excretion following an oral salt load of 0 1 g/kg body weight glomerular filtration rate and hematocrit in preterm infants of various gestational ages. All studies performed 1–7 days after birth The figure also shows the value found in full-term infants. Range bars represent standard error of mean (S E M).

age the neurological maturity and external features were examined (1 19 22, 34) Each infant accepted for the study was appropriate in weight and length for gestational age (32) The infants were divided into 3 groups according to gestational age Group I 29–33 weeks 15 infants. Group II 34–35 weeks 16 infants Group III 36–37 weeks 13 infants Group IV 38–42 weeks 23 infants The results in 20 of these full-term infants of Group IV has been reported in a previous communication (3)

The preterm infants were studied during the first week after delivery (18 infants) –3 weeks after delivery (15 infants) and at a postmenstrual age of 40 weeks (16 infants) Breast milk or cow s milk formulas (Baby Semp 1 or Milkotal) were fed to all infants studied The daily caloric intake was 120–130 kcal/kg body weight The studies were performed in the nursery and the babies were in their own incubator or bed The body temperature did not change and there were no signs of discomfort Informed parental consent was obtained in all cases studied

All studies were carried out during standardized fluid expansions. The infants were fed by stomach tube During the entire course of the study they were given a standardized formula (Baby Semp 1) or breast milk diluted 1 3 It was given an amount corresponding to 2% of the body weight during the first hour followed every 30 minutes by an amount of 0 5% of the body weight The salt load was given after 90–120 minutes as a 1% saline solution Sodium chloride in an amount of 0.12 g/kg body weight was dissolved in the diluted formula Urine was obtained by spontaneous voidings which in the very preterm infants occurred at 10–120 minute intervals During the latter part of the study i e after the salt load had been given GFR was determined with single injection technique (28) in 17 preterm infants and in 3 full-term infants. Inulin was used as indicator substance 0 75 ml (10% Inulin Laevasar Gesellschaft) per kg body weight was given as a single intravenous injection in a scalp vein Capillary blood was taken every 5 minutes during the first 30 minutes after the injection then every 10–15 minutes during the following 55 minutes.

The sodium concentration in serum and urine was analyzed by a flame photometer (Eppendorf) Inulin in blood was determined according to Heyrovsky (16). Osmolality in blood and urine was determined cryoscopically with the aid of a Knauer osmometer Serum albumin was determined by a refractrometeric method Hematocrit in capillary blood was estimated in glass capillaries which were centrifuged at 10000 rpm for 5 minutes.

Students *t*-test has been used in statistical analyses.

RESULTS

All infants studied responded to the oral sodium load with an increased urinary sodium excretion In the newborn full-term infants the pattern of the responses was rather consistent The hourly sodium excretion increased during the first hour after the load had been given and was then fairly constant The pattern of the response in older children (15) was similar to that observed in full-term infants The magnitude of the response was however much larger in the older children In the very preterm infants the response to the oral sodium load was more variable Dif

[1] Baby Semp 1 (Semper) sodium content 6.5 mEq/l Milkotal (Findus) sodium content 8.7 mEq/l pooled human breast milk, sodium content about 7 mEq/l

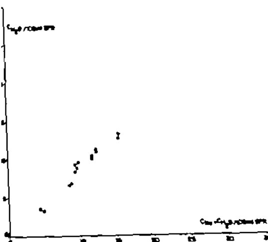


Fig. 3. The relationship between the sum of sodium and free water clearance ("distal tubular Na delivery") and free water clearance ("distal tubular Na re-absorption"). ● Values obtained in 5 newborn full-term infants. ○ values obtained in 5 preterm neonates 29–35 weeks of gestational age.

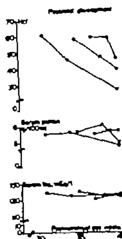


Fig. 4. Postnatal values of serum sodium, serum protein and hematocrit in preterm infants. Each value represents the mean of 3–7 investigations.

ferent patterns of response are demonstrated in Fig. 1. In some infants there was one short but intense period of natriuresis, in others there was one or several short periods of higher sodium excretion. Between these periods the excretion was low. This might be due to greater variations in the time between each voiding and incomplete emptying of the bladder. Errors of this type can be expected to be less if more collecting periods are included. It therefore seems correct to represent the urinary sodium excretion for each infant as the average hourly sodium excretion calculated from all urinary samples obtained between 1 and 5 hours after the sodium load had been given.

First week after birth

Fig. 2 shows the average urinary sodium excretion, the GFR and the hematocrit in infants of different gestational ages 1–7 days after birth. The hematocrit was the same in the 4 different groups. GFR was higher in infants with a gestational age above 36 weeks than in those with a gestational age of 29–35 weeks. The difference was of border-line significance ($p=0.1$). The natriuretic response was significantly higher in infants of 29–35 weeks gestation than in infants of 36–37 weeks gestation and full-term infants ($0.005>p>0.001$). The natriuretic response in the preterm infants is, however, still five to ten-fold lower than the natriuretic response observed in children 8–14 years old (5).

It is generally accepted that the diluting capacity is a function of distal tubular sodium re-absorption (21, 31). The diluting capacity is most often measured as free water clearance. A characteristic relationship normally exists between the diluting capacity and the distal tubular sodium delivery, which can be given as the *sum of free water clearance* (C_{H_2O}) and sodium clearance (C_{Na}). In Fig. 3 the relationship between free water clearance (C_{H_2O}) and distal tubular delivery ($C_{H_2O}+C_{Na}$) is demonstrated and compared with that found in full-term infants. The relationship is just about the same in both groups of infants, though the diluting capacity seems to be somewhat better in the preterm infants.

Postnatal development

Fig. 4 shows the postnatal changes of hematocrit and the serum concentrations of albu

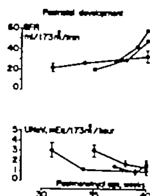


Fig 5 Postnatal development of the natriuretic response to an oral salt load of 0 17 g/kg body weight represented as mean values of the average hourly urinary sodium excretion Mean values of glomerular filtration rate during postnatal development are also represented. Range bars represent standard error of mean (S E M)

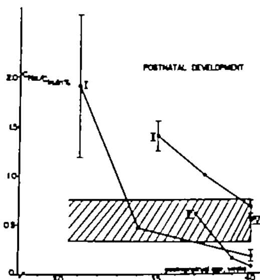


Fig 6 Postnatal changes in fractional sodium excretion represented as mean values. Range bars and the hatched area represent standard error of mean (S E M)

min and sodium in preterm infants of different gestational ages The infants are followed until a postmenstrual age of 40 weeks There was as expected a general fall in the hematocrit (15) which was most pronounced in the most preterm group The well known fall in serum albumin concentration (33) was observed The serum sodium concentration remained constant

The postnatal development of the urinary sodium excretion and GFR in infants of varying gestational ages is demonstrated in Fig. 5 The GFR increased in all groups In the very preterm infants the GFR increased from 22 ml/min to 32 ml/min/1 73 m² body surface In infants with gestational ages of 34–35 and 36–37 weeks the GFR increased to 48 ml/min and 58 ml/min/1 73 m² body surface respectively In preterm infants the GFR was consistently higher at a postmenstrual age of 40 weeks than in newborn full term infants The difference was found to be most pronounced in infants born after 34–37 weeks gestation The determinations of GFR were however too few to allow statistical analysis

The response to an oral salt load develops in a characteristic way during the postnatal period In the very preterm newborn infants the urinary sodium excretion following a salt load was comparatively high During the postnatal period the natriuretic response was reduced to the same level as in newborn full-

Table 1 *The diuretic response in infants of different postmenstrual age*

Gestational age (weeks)	Postnatal age 0–1 week	Postnatal age 2–3 weeks	At expected term
Group I 29–33	157 4±13 0* n=5	149 1±39 6 n=6	189 9±49 8 n=7
Group II 34–35	159 0±27 9 n=7	164 8±35 1 n=6	210 8±43.5 n=4
Group III 36–37	133 6±37.2 n=6	180.3±56 7 n=3	188 6±71.8 n=5
Group IV 38–42			168 1±53.3 n=23

The values represent mean±1 S.D and are expressed in ml/1 73 m²/hour

erm infants. In infants born after more than 6 weeks gestation the response was about he same as in newborn full-term infants

The postnatal changes in the relationship etween sodium excretion and GFR are demnstrated in Fig. 6 The urinary sodium ex retion in percentage of filtered sodium (C_{Na}/C_{In}), the so-called fractional sodium excre ion, is given and related to postmenstrual and postnatal ages The sodium excretion in elationship to the GFR is highest in the infants with the lowest postmenstrual age With longer gestation as well as with increasing postnatal age the fractional sodium excretion decreases

The diuretic response following the salt load is shown in Table 1 No statistically significant difference could be found between the different groups except in Group II where an almost significant difference exists between first week and at expected term

DISCUSSION

As expected (4 36) glomerular filtration rate (GFR) was found to be low in preterm infants even when correlated to body surface The difference in GFR between preterm and full-term infants correlates well with the results of histopathological studies (22 25) according to which the formation of new glomeruli is not complete until the 36th gestational week. Another factor that could contribute to the low GFR is the low hydrostatic pressure (30) *In view of the low GFR in preterm* infants the relatively high natriuretic response following a salt load might be somewhat surprising. It should however be noted that the urinary sodium excretion is not only a function of GFR but also of tubular re absorption. When rapid changes in salt balance occur—as for instance by extracellular volume expansion—the resulting diuresis is more an effect of inhibition of tubular sodium re-absorption than of an increase of the filtered load (11) Some of the factors that influence tubular sodium re-absorption are intrarenal physical forces such as hydrostatic and oncotic pressure between peritubular capillary and renal interstitium (6 20) Newborn infants have a low hydrostatic pressure and a high hematocrit which would both enhance sodium re-absorption (29) On the other hand serum albumin concentration is low which should depress sodium re absorption Presently there is nothing that indicates that a hormone acting on tubular sodium re-absorption would be responsible for the sodium retention in early infancy The aldosterone secretion rate has been shown to be low during the first week after birth (35)

The developmental stage of renal function can be expected to be correlated to the anatomical development of the kidney (24) Micro-dissection studies from young stillborn fetuses and infants have revealed that the vascular supply develops from the inner medulla to the outer cortex (21) The glomeruli of the juxtamedullary zone are the first ones to develop If one assumes that the functional and anatomical developments run parallel the major part of renal function would be carried out in the juxtamedullary nephrons in very young infants Since extracellular volume expansion inhibits sodium re-absorption in superficial nephrons but not in juxta medullary nephrons (18) thus centrifugate development of nephrons might be one factor that could explain sodium retention in very young infants It does not however explain the difference between preterm and full-term infants

Micro-dissection studies in infants (14) and newborn dogs (17) have revealed a glomerular-tubular imbalance with larger glomeruli and smaller tubular mass as compared with older children and adult dogs It seems likely that, in preterm infants the tubular mass is even smaller than in term infants and thus the glomerular-tubular imbalance even larger The relatively high fractional sodium excretion in the very preterm infants might thus be due to the fact that the tubular surface area for re-absorption is inadequate.

The free water clearance data strongly suggest that the sodium pump functions optimally even in very preterm infants The lowest osmolalities observed in neonates 25–35 mOsm/l are much lower than those observed in older children and adults in this laboratory This indicates that sodium can be reabsorbed against a high transtubular concentration gradient The function in each tubular unit in the very preterm neonates should thus be as good as in newborn full term infants

In addition some extra-renal factors may also account for the higher sodium excretion in preterm than in full term infants Preterm infants have a larger extracellular volume (10) and a higher relative total sodium content (23) than full-term infants Both those conditions are known to enhance the urinary sodium excretion (7 11 12 26 36)

The results of the studies of GFR and urinary sodium excretion in preterm infants of various postnatal ages give some views on the influence of extra-uterine life on the development of renal function At a postmenstrual age of 40 weeks GFR was found to be about the same in very preterm infants as in newborn full-term infants In infants with 34–37 weeks gestation the GFR was somewhat higher at expected term than in newborn full term infants Since the observations are very few they do not allow of any definite conclusions on a postnatal acceleration of the GFR in those groups In case an actual postnatal acceleration exists it seems unlikely that this postnatal acceleration of the development would be due to high protein intake or a high mineral load (9 13) since in our studies the protein intake was only 2 2 g/100 Cal and the sodium intake was fairly low (1 0 mEq Na^+/100 Cal)

The results from the present study are well compatible with the hypothesis that extrauterine life has only minor influence on the development of the control of sodium homeostasis The developmental changes in glomerular-tubular balance discussed above could explain the reduction in urinary sodium excretion during early postnatal development among preterms It should be noticed however that also environmental factors may contribute to the reduction in the natriuretic response to an oral salt load One such factor might be that breast-fed newborn infants have a very low sodium intake So far unpublished observations from this laboratory have demonstrated that when salt intake is restricted in older children the natriuretic response to an oral salt load is drastically reduced or even abolished

In newborn full-term infants an inverse relationship has been demonstrated between the hematocrit and the natriuretic response to the oral salt load (3) Since polyglobulia has been shown to accelerate the renal tubular sodium reabsorption by the influence of intrarenal physical forces (8 29) a high hematocrit was supposed to be one of the factors contributing to salt retention in very young infants In the present study it was found that in preterm infants the natriuretic response to an oral salt load was still low 4–10 weeks after birth that is at an age when the hematocrit is at a minimum level Thus polyglobulia cannot be a major factor responsible for the salt retention in the early postnatal period

Renal excretory capacity is extremely low in preterm and full-term infants compared with older children and adults The fact that GFR is even lower in preterm neonates than in full-term newborns has obvious clinical implications as regards the prescription of drugs which are excreted by the kidneys In preterm neonates the basal sodium excretion seems to be set at a higher level than in full term infants The limits for salt tolerance are thereby still more narrow in the preterm infant since the risks of giving too small amounts of sodium will also have to be considered In very preterm infants extra-uterine life seems to have little influence on the development of GFR as well as on the development of the response to an oral salt load

When calculating the sodium tolerance in a preterm or full-term infant during early infancy postmenstrual rather than postnatal age has to be considered

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ARGININOSUCCINIC ACIDURIA

Report of Three Cases and the Effect of High and Reduced Protein Intake on the Clinical State

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ABSTRACT Hambræus, L. Hardell, L. I., Westphal, O., Lorentsson, Rosa and Hjorth Gunilla (Department of Nutrition, Institute of Medical Chemistry and Department of Pediatrics, University Hospital University of Uppsala, Sweden). Argininosuccinic aciduria. Report of three cases and the effect of high and reduced protein intake on the clinical state. Acta Paediatr Scand, 63: 525, 1974.—Three girls with argininosuccinic aciduria (ASA) and hyperammonemia are reported. Two of them are siblings. They showed the typical clinical findings of ASA, i.e. developmental retardation, cerebellar ataxia and short brittle hair as well as episodes of changed behaviour or unconsciousness. The dietary history revealed that they all spontaneously had chosen low-protein diet, thus lowering the risks of hyperammonemia. The effect of different protein intake was studied by means of short time metabolic balance studies. High protein intake provoked clinical signs of encephalopathy objectively registered EEG changes, most probably due to subsequent hyperammonemia During 16–17 months follow-up on reduced protein intake—the easiest way to minimize the risks of hyperammonemia—no acute attacks of ammonia intoxication were observed but unfortunately the patients did not gain sufficiently in length.

KEY WORDS: Argininosuccinic aciduria, hyperammonemia, protein intake

Argininosuccinic aciduria (ASA) represents an inborn error of the metabolism which affects the Krebs-Henseleit urea cycle and was first described in 1958 (2) Since then about 20 further cases of ASA have been described in the literature (3 5 7 8 9 22 29 31 32) However no cases seem to have been diagnosed in Scandinavia until these cases were first described (39)

The biochemical defect results in an accumulation of arginino-succinic acid (AS) and citrulline in blood urine and cerebrospinal fluid. Marked hyperammonemia has been reported in some cases of ASA (31) whilst in other cases only intermittent hyperammonemia has been reported (29) or as in most cases no elevation of the blood ammonia

The present report concerns the findings in three cases of ASA which were diagnosed by means of a metabolic screening examination carried out on specimens of urine from patients with mental and neurological disturbances of unknown etiology (15) The possibility of provoking the clinical symptoms by a high protein intake was studied as well as the short-term and long-term effects of a reduced protein diet.

CASE REPORTS

Case I M K born February 15 1961

A girl, eldest among three siblings parents healthy and not related A brother born in 1963 is healthy and a sister born in 1965 is patient no 2.

Pregnancy and delivery were normal. Birthweight 3760 g. She learnt to walk at 1½ years of age and

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hair in addition to the metabolic abnormalities

METHODS

Quantitative amino acid analyses were performed by means of an automatic amino acid analyzer (BIOCAL BC 200). Deproteinization was accomplished by adding 100 mg of solid sulphosalicylic acid to each ml sample of plasma or cerebrospinal fluid. After centrifugation the supernatants were kept frozen at $-20°C$.

Creatinine-nitrogen and *urea nitrogen* in blood and urine were determined by means of the Technicon Auto-Analyzer® according to their methods nos N-11b and N 1c.

Total nitrogen was determined in blood, urine, faeces as well as in the double portions of the food by means of the Tecator Digestor® and the Technicon Auto-Analyzer® as follows: 1 ml of the blood and ml of the urine was digested to clearness for 20 minutes at 370°C together with 3 ml of a mixture of sulphuric and phosphoric acid (5 parts phosphor acid p.a. and 95 parts sulphuric acid v/v and 1 tablet of Kjeltabs Auto®" which contains 1.5 g potassium sulphate and 0.0075 g selenium). 1.5 ml of hydrogen peroxide was added carefully. Faeces and the double portions of food were homogenized in a homogenizer and an aliquote was taken using the equipment described by Isaksson (18). 4-5 g of the homogenate was digested to clearness for about 60 minutes at 370°C together with 6 ml of the sulphuric acid phosphoric acid solution described above and 2 Kjeltabs Auto tablets. 3 ml of hydrogen peroxide was added carefully.

After digestion all the samples were diluted to give a final concentration of about 30–100 ppm nitrogen and analysed by means of the indophenol reaction according to Kloosberg (30).

Ammonia levels in blood were estimated according to a modification of the method described by McCullough (27) as follows:

Blood specimen was taken from the cubital vein and the first 10 ml of blood were collected in heparinized tube for amino acid analyses. Another 2 ml of blood was taken without stasis and immediately pipetted into 10 ml tube containing 1 ml of sodium tungstate concentrate and 1 ml of 1 N sulphuric acid was added. The tubes were centrifuged at 3 000 rpm for 10 minutes after thorough mixing. The supernatant was kept frozen until analysis which was performed within 30 minutes. To 0.5 ml of each supernatant 0.5 ml of distilled water, 0.5 ml of solution no 1 (containing phenol, 5 g, and sodium nitroprusside 25 mg, dissolved in 100 ml of distilled water) and 0.5 ml of solution no (containing sodium hydroxide [illegible]5 g, disodium hydrogen orthophosphate $Na_2HPO_4 \cdot 7H_2O$ 20.1 g, and 5 ml of sodium hypochlorite made up to 100 ml with distilled water) were added. The mixture was carefully mixed and the tubes were incubated at 40°C for 15 minutes. Absorbance was read at 625 nm in a 10 mm cuvette. Each time standards containing 50 µg, 100 µg, 200 µg and 400 µg of ammonia nitrogen (as ammonium sulphate) were run and a standard calibration curve was drawn.

Estimations of the urinary excretion of AS were performed by means of the amino acid analyser. In order to convert the free AS to the more stable anhydrides the urinary samples were boiled for 2.5 hours (3).

Statistical analyses were performed using conventional methods. Significances of differences of the means were determined using the Student's *t*-test. The levels of significances used were as follows: almost significant ($0.01<p\leq 0.05$), significant ($0.001<p\leq 0.01$) and highly significant ($p\leq 0.001$) and indicated on the tables and figures by and ** respectively.

METABOLIC STUDIES

Studies of the effect of protein intake were performed as conventional nitrogen balance studies during five different periods. The patients were kept at a metabolic ward during these studies. Data about the diets given during these five separate periods are presented in Table 1.

The protein intake was calculated according to the conventional food tables (1) as well as determined by means of the double portion technique. The double portions were collected for each day in special bottles and frozen at $-20°C$.

Fig. 1 shows that the correlation between the calculated protein intake according to food tables and the protein content analyses in the double portions was very close and highly significant. However the absolute values of the calculated protein intake were somewhat higher than the analysed one but these differences were not significant.

Table 1. *Metabolic study with diets of different protein content*

Period no	Case no 1 (M. K.) Kcal/kg	Protein g/day	g/kg	Case no (A. K.) Kcal/kg	Protein g/day	g/kg	Case no 3 (A. O.) Kcal/kg	Protein g/day	g/kg
1	46	1 0	0.46	74	1 5	0 72	63	17 5	0 93
	47	4 6	0 18	74	4 7	0 27	63	4.5	0..4
3	38	47 8	1 85	58	44 4	2.50	63	90.3	2 63
4	58	5 3	0.20	75	5	0.30	35	3 0	0.16
5	40	8 4	0.32	68	13 5	0.75	66	10.5	0.52

started to talk at ? At the age of 8 years she still had an undeveloped speech Her behaviour has always been infantile unconcentrated and volatile for her age At the age of 10 years she spoke almost normally and was trained at a school for mentally retarded knew a few letters but was not able to read

Repeated grand mal fits occurred during an infection with slight to moderate fever when 8 months old Since then she has had altogether 15–20 episodes with fits mainly grand mal not always combined with infections. In 1966 one episode occurred with high fever generalized stiffness and unconsciousness which was thought to be due to acute encephalitis Her CSF showed a normal cell count and a normal protein level EEG was severely abnormal with slow activity and unspecific asymmetric changes She awoke after one day and then recovered rapidly EEG became normalized (but not normal) within 10 days. Furthermore she had 5–10 episodes of –3 days duration when she was mentally changed just as if drunk These episodes also occurred in connection to infections.

Physical examination In 1968 her hair was abnormally brittle and not more than 5 cm long (never cut!) Her teeth had a marked degree of caries but no enamel hypoplasia She was clumsy and infantile in all motor performances Motor age examination gave scores of 50–55 months for both upper and lower extremities (chronological age 9? months) She performed at a mental level of about 4 years her speech development lay on a level of about 3 years She was unconcentrated and volatile but kind and smiling no normal rejection of strangers Her orientation in space was poor and she had a tendency to perseverate In February 1971 her motor functions were still rather clumsy with a dysharmonic way of running and showed the clinical picture of cerebellar ataxia She had obvious difficulty in buttoning her clothes. No other neurological defects were revealed.

EEG was found to be abnormal with pronounced generalized changes of unspecific type In addition several epileptogenic discharges with variable localization occurred EMG and nerve conduction velocity rates were normal.

X ray The skull and the extremities were normal. The skeletal age corresponded to the chronological.

Laboratory findings Routine blood CSF and urine values were all normal. Serum electrophoresis revealed an increase in α_2-globulins. Serum bilirubin thymol alkaline phosphatases GOT and GPT were normal An intravenous galactose tolerance test (29) was normal ($t_{\frac{1}{2}}$ was 10 minutes normal less than 17 minutes) Fractionated plasma lipid pattern was normal Creatinine was 0 8 mg/100 mg.

Case 2 A K born March 2 1965

A younger sister of patient no 1 Pregnancy and delivery were normal Birthweight 4400 g. No prenatal anomalies The psychomotor development was retarded and very similar to her sister s. She had never had any convulsions, but in October 1970 during a period of fever she became semicomatous for about 4 hours The episode was similar to those of her sister

Physical examination (February 1971) Her hair was only ? cm long (never cut!) dry and brittle Curious changes were found in several teeth She was slightly infantile in her motor performances in relation to her age She functioned at a mental level of about 4 years (Her behaviour was rather similar to that of her sister) She had a mild cerebellar ataxia but no other neurological defects were revealed

In January 1968 EEG showed shifting and bilateral synchronous discharges over both hemispheres A progress was shown on reexamination in 1971 EMG and nerve conduction velocity rates were normal.

X-ray The skull spine and extremities were all normal The skeletal age corresponded to the chronological.

Laboratory findings Routine blood and urine values were all normal The CSF protein level was 65 mg/100 ml. CSF electrophoresis revealed an increase of α_2-globulins. Serum bilirubin 0.4 mg/100 ml Thymol 8 5–0.7 U alkaline phosphatases 4.5 BLU,GOT 90 and GPT 70 KWU An intravenous galactose tolerance test (29) was normal ($t_{\frac{1}{2}}$ 7 5 min, normal less than 17 minutes) Fractionated plasma lipid pattern showed an increase of triglycerides (total lipids 983 mg/100 ml) Creatinine was 0 9 mg/100 ml.

Case 3 A O born September 25 1965

A girl eldest of two siblings both parents healthy and not related Her sister born in 1970 is healthy

Pregnancy and delivery were normal The birth weight was 3 560 g. No prenatal anomalies She learnt to walk at 1½ years and started to talk at 2½ years. At the age of 4 years her mental development was that of a normal ? year-old child She was described as unconcentrated and volatile for her age

When 16 months old an episode of muscular hypotonia and unconsciousness was noticed EEG showed epileptic episodes with variable localization Since then she has had about 10 episodes of similar type often in connection with fever

Physical examination Her movements were stiff and clumsy and motor age examination gave a score of about 3 years She had an ataxia of cerebellar type A moderate mental retardation was noted her speech was infantile and she was rather unconcentrated without normal rejection of strangers Her hair was somewhat brittle but no defects were seen in her teeth

EEG was found to be pathological with epileptogenic spikes or spike and wave discharges of variable localization

Laboratory findings Routine blood, CSF and urine values were all normal Serum bilirubin 0 6 mg/100 ml thymol 0 6 U alkaline phosphatases 3 3–3 6 BLU GOT 4 and GPT 29 KWU Creatinine was 0 8 mg/100 ml.

In summary all three patients showed a developmental retardation ataxia of cerebellar type fits or disturbed cons

hair In addition to the metabolic abnormalities

METHODS

Quantitative amino acid analyses were performed by means of an automatic amino acid analyzer (BIOCAL BC 200). Deproteinization was accomplished by adding 100 mg of solid sulphosalicylic acid to each 2 ml sample of plasma or cerebrospinal fluid. After centrifugation the supernatants were kept frozen at −20°C.

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Estimations of the urinary excretion of AS were performed by means of the amino acid analyser In order to convert the free AS to the more stable anhydrides the urinary samples were boiled for _5 hours (3).

Statistical analyses were performed using conventional methods. Significances of differences of the means were determined using the Student s *t*-test. The levels of significances used were as follows almost significant ($0.01<p\leq0.05$) significant ($0.001<p\leq0.01$) and highly significant ($p\leq0.001$) and indicated on the tables and figures by and ** respectively

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Table 1 *Metabolic study with diets of different protein content*

Period no	Case no 1 (M K)			Case no (A. K.)			Case no 3 (A. O)		
	Kcal/kg	Protein g/day	g/kg	Kcal/kg	Protein g/day	g/kg	Kcal/kg	Protein g/day	g/kg
1	46	1 0	0 46	74	1_5	0 7?	63	17.5	0 93
	47	4 6	0.18	74	4 7	0 77	63	4.5	0. 4
3	38	47 8	1.85	58	44 4	90	63	50.3	_63
4	98	5 3	0.20	75	5_	0 30	35	3 0	0 16
5	40	8 4	0 37	68	13 5	0 75	66	10 5	0.52

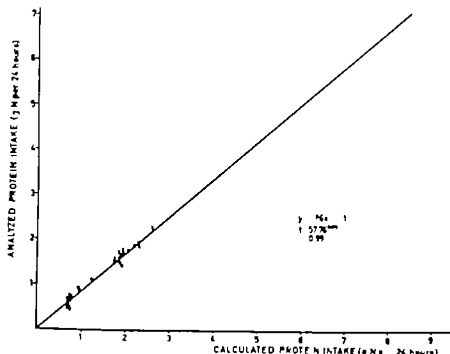


Fig 1 Correlation between the analysed and calculated protein intake during the metabolic balance studies.

During the first four balance studies the short-term effects of different protein intake were studied The caloric and protein intake during the first period was supposed to be identical with that in the diet spontaneously chosen by the patients It was composed after a careful recall with the mothers

The diets given during the fifth balance period simulated the reduced protein diet (about 0.5 g/kg body weight) given during a follow-up period of 16–17 months in order to keep blood levels of AS and ammonia within a normal range The effect on growth and development was studied after this follow up period.

Blood samples for assay of amino acids were taken in the morning using heparinized Vacutainer® tubes before the first and fifth period and at the end of each period The specimens were immediately centrifuged and the plasma deproteinized

Urine was collected in 24 hour samples. During the collection the specimens were kept in a freezer at −20°C as well as when the collection was completed Before analysis the urine sample was carefully mixed.

Faeces samples were collected in special bottles during each period in one portion Each period which comprised 3 to 4 days, was indicated by Carmine which was given orally in the beginning of each new period After collection the samples were kept frozen at −20°C until analysis

Electroencephalograms were taken during the first third and fifth periods

RESULTS

As seen in Tables 2 and 3 the three patients showed almost the same biochemical abnormalities

The pathological *plasma aminogram* (Table 2) was characterized by a more than tenfold increase of the citrulline concentration and a high concentration of AS which does not occur in normals The glutamine level was also frequently increased in the plasma representing 2 to 5 times the normal values The plasma level of proline was increased twofold on almost all occasions furthermore the glycine alanine and serine were occasionally increased 1–2 times the normal values Somewhat low values were found with respect to the essential amino acids valine isoleucine and leucine The arginine value in the plasma was usually below the normal range and sometimes only traces of arginine were found The ornithine level also showed values somewhat below the normal range on most occasions The plasma amino acids were completely within the normal limits in the specimens analysed from the healthy brother of patients 1 and 2

Amino acid analyses of the *cerebrospinal fluid* (Table 3) were performed in cases 1 and 2 (in case no 2 on two separate occasions) but not in their healthy brother nor in case no 3 It was seen that the AS and citrulline levels in the cerebrospinal fluid

Table 2. *Amino acid concentrations in plasma*

Values are given as µmoles per litre

Amino acids	Case no. 1 (M. K.) Oct. 1968	March 1971	Aug. 1972	Case no. 2 (A. K.) Oct. 1968	Nov 1970	March 1971	Aug. 1972	Case no. 3 (A. O.) Sept. 1970	May 1971	Aug. 1972	Normals* Mean ±S.D.
Taurine	83	58	57	39	149	3	4	76	75	37	38± 21
Aspartic acid	8	•	46	9	•		47		76	22	10± 6
Threonine	64	53	75	51	107	46	73	48		67	92± 40
Serine	•	11	177	80	339	147	136	139	703	180	102± 43
Asparagine		•	97	•		•	68			77	44± 16
Glutamine	800	659	4066	586	1968	1177	3181	1 19	1675	949	678±244
Proline	196	138	185	45	257	124	173	14	12	706	139± 51
Glutamic acid	107	66	123	48	63	53	43	125	75	29	73± 73
Citrulline	18	119	148	321	157	1 4	49	779	258	285	79± 1
Glycine	173	223	289	189	700	709	336	772	411	462	216± 77
Alanine	214	348	223	145	113	358	315	182	479	510	298± 79
Valine	144	106	179	193	108	118	103	156	135	107	21 ± 83
Cystine	97	57	24	66	90	50	13	73	73	39	37± 11
Methionine		8			8			14	16	11	21± 8
Isoleucine	34	28	45	65	42	4	4	33	26	28	65± 79
Leucine	76	71	79	113	51	53	49	68	58	52	118± 51
Tyrosine	71	41	30	57	29	33	24	31	38	43	6_± 26
Phenylalanine	30	39	56	43	28	38	24	30	35	28	48± 16
Ornithine	28	30	40	31	57	26	38	37	46	46	71± 22
Lysine	122	101	38	209	184	59	44	177	161	77	161± 53
Histidine	89	97	170	102	117	88	701	131	135	102	97± 39
Arginine	16	79	Trace	Trace	49	20	23	37	30	Trace	77± 32
A.S.A.	n.d.	99	270	n.d.	173	137	339	225	196	406	–

Holmgren (16). • Not satisfactorily separated

Table 3. *Amino acid concentrations in the cerebrospinal fluid*

Values are given as µmoles per litre

Amino acids	Case no. 1 (M. K.)	Case no. 2 (A. K.) I	II	Normals*
Taurine	3.5	Trace	5.6	
Aspartic acid	Trace	Trace		
Threonine	13.8	7.5	22.8	16.5–121
Serine				194–865
Glutamine	694	•	1500	194–865
Proline	–	–	–	0–5.8
Glutamic acid	31.8	25.8	19.2	2.4–24.6
Citrulline	22.4	19.9	17.0	1.1–4.5
Glycine	5._	3.9	12.8	4.0–10.7
Alanine	13.9	8.4	40.4	11.7–44.6
Valine	10		6.5	7.4–28.2
Cystine	17.6	8.4	–	0–1.4
Methionine				0.5–7.5
Isoleucine	Trace	3._	3.5	3–8.6
Leucine	Trace	6.3	6.5	6._–22.2
Tyrosine	10.8	4.7	7.1	3.0–24.3
Phenylalanine	5.8	4.3	4.9	1.6–27.4
Ornithine	–	Trace	–	3.7–10.2
Lysine	23.5	20		6.1–33.4
Histidine	21.4	15.6		6.2–23.8
Arginine	Trace	Trace		8.0–29.9
A.S.A.	12	118.7	252.1	0

Dickinson & Hamilton (13).
• Not satisfactorily separated.

were extremely high but also the cystine concentration was higher than that reported in normals. The glutamine concentration was about twice the upper normal limit in case no. 2 whilst it was within the normal range in case 1. It should be observed that the ornithine as well as the arginine concentration of the cerebrospinal fluid were far below the normal limit.

The *urinary amino acids* were found to be within the normal limit and the main pathological finding was the very pronounced excretion of AS which was found to vary between 640 and 1450 mg per day in case no 2, between 1550 and 2310 mg per day in case no. 1 and about 3840 mg per day in case no. 3. Citrulline was found in the urine in all cases otherwise no other major abnormalities were found in the urinary amino acid pattern.

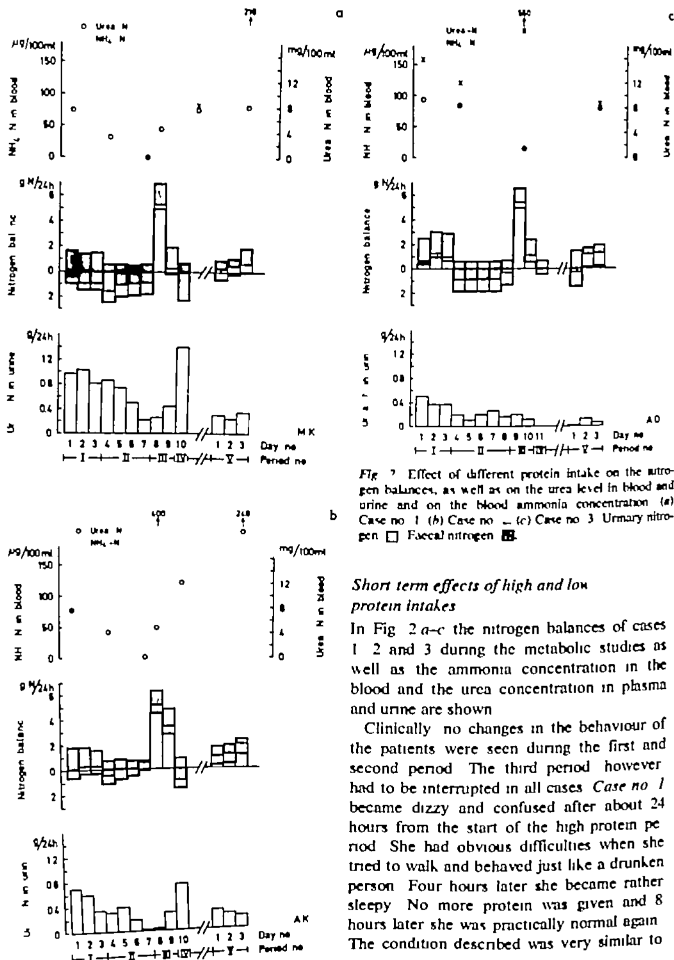


Fig 2 Effect of different protein intake on the nitrogen balances, as well as on the urea level in blood and urine and on the blood ammonia concentration (*a*) Case no *1* (*b*) Case no 2 (*c*) Case no 3 Urinary nitrogen ☐ Faecal nitrogen ▦.

Short term effects of high and low protein intakes

In Fig 2 *a–c* the nitrogen balances of cases 1 2 and 3 during the metabolic studies as well as the ammonia concentration in the blood and the urea concentration in plasma and urine are shown

Clinically no changes in the behaviour of the patients were seen during the first and second period The third period however had to be interrupted in all cases *Case no 1* became dizzy and confused after about 24 hours from the start of the high protein period She had obvious difficulties when she tried to walk and behaved just like a drunken person Four hours later she became rather sleepy No more protein was given and 8 hours later she was practically normal again The condition described was very similar to

her spontaneous episodes according to her mother who was present An EEG showed an increasing amount of slow and irregular discharges when compared with earlier recordings *Case no 2* became dizzy and anxious about 30 hours from the start of the high-protein period and sleepy about 5 hours later In general she showed the same picture as her sister but to a lower extent. EEG showed slight progress of irregular unspecific discharges when compared with earlier investigations *Case no 3* became dizzy and anxious about 28 hours from the start of the high-protein diet and vomited 4 hours later She had obvious difficulties when she tried to walk A few hours later a marked increase in her ataxia was noted and she was very very sleepy EEG during the high protein diet showed a considerably increase in slow activity irregular discharges when compared to an EEG-recording 6 days earlier

The effect of the protein intake on the plasma aminogram in the patients are shown in Fig. 3 *a–c* As seen in the figure all patients showed the same changes characterized by a marked increase of proline citrulline valine isoleucine leucine tyrosine and AS during the high protein intake When a low protein diet was given however there was a marked increase in the plasma levels of glycine and alanine

Effects of reduced protein intake during 16–17 months follow-up

During the follow-up period of 16–17 months none of the three patients had any attacks of convulsions unconsciousness or somnolence not even during febrile illnesses Their psychomotor development improved and the pathological changes of the EEGs decreased However all of them still showed a mild cerebellar ataxia. All patients decelerated in growth Laboratory findings were in the main unchanged No changes in the serum protein electrophoresis serum bilirubins alkaline phosphatases GOT or GPT were registrated Folic acid and B_{12} vitamin levels in the serum were also normal Serum iron levels were within or just below the normal limit Ammonium levels are shown in Fig 2.

Case 1 (who had the lowest protein intake) developed keratotic changes of the skin of the palms and the soles and her hair became even more brittle (Fig. 4) She did not gain in length at all and her weight gain was only 0 4 kg.

Case 2 had very mild keratotic changes of the skin and her hair thickened She gained 2 cm in length and put on 0 9 kg in weight.

Case 3 during this follow-up period once lost her hair completely and also got changes of the skin of the same type as case no 1 Without any obvious changes in the protein intake she improved concerning the growth of her hair and the changes of her skin Her lenght was unchanged but she put on weight 1 2 kg.

DISCUSSION

The biochemical findings and the clinical picture in our three patients with ASA are very similar to those earlier described in this disease and the only way to verify the diagnosis of ASA still seems to be to reveal the urinary excretion of AS or the increased plasma levels of AS or citrulline (37) This can only be made by means of chromatographic methods

The findings in our patients seem to indicate that the rapid changes in their clinical state and the serious signs where the cerebra[l] damage and intoxication are dominant ar[e] due rather to the hyperammonemia *per se* than to the pathological blood levels of cer[tain] amino acids. Consequently in our opin[ion], all efforts should be made to avoid am[monia] intoxication. The easiest way o[f] minimizing the risks of hyperammonemi[a] seems to be to reduce the protein intake As shown in our studies this also represent[s] an effective way in avoiding hyperam[mo]nemia, since during the follow-up perio[d] our patients had no serious fits and were [...]

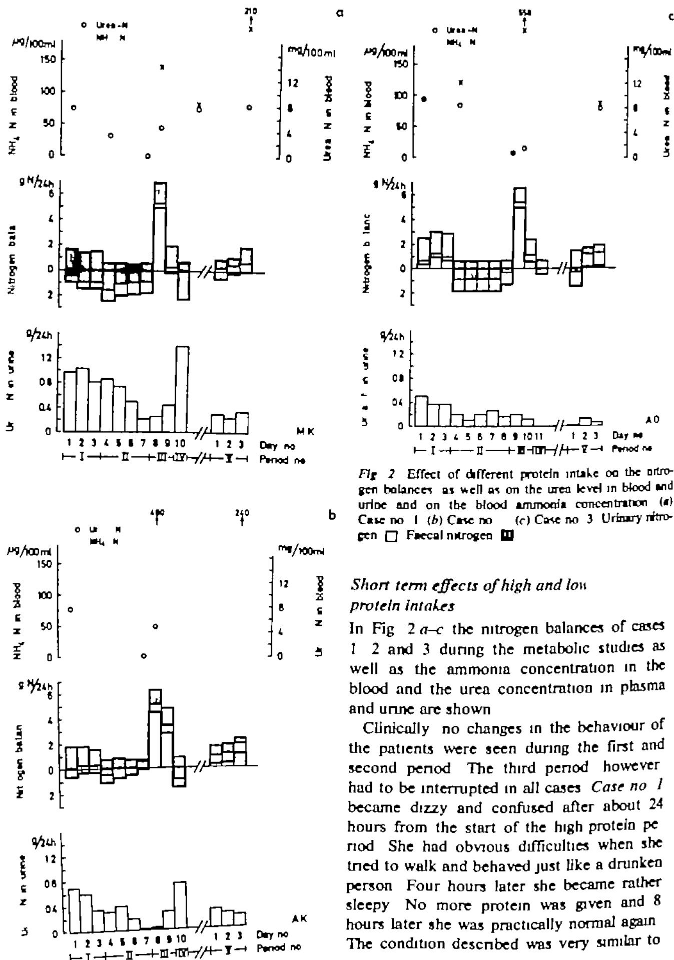


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The findings in our patients seem to indicate that the rapid changes in their clinical state and the serious signs where the cerebral damage and intoxication are dominant are due rather to the hyperammonemia *per se* than to the pathological blood levels of certain amino acids Consequently in our opinion all efforts should be made to avoid ammonia intoxication. The easiest way of minimizing the risks of hyperammonemia seems to be to reduce the protein intake As shown in our studies this also represents an effective way in avoiding hyperammonemia since during the follow-up period our patients had no serious fits and were in

Fig. 3. Effect on the plasma aminogram of different protein intake. (a) Case no. 1. (b) Case no. – (c) Case no. 3.

c

TAU THR SER GLU-NH2 PRO GLU CIT GLY ALA VAL CYS MET ILEU LEU TYR PHE ORN LYS HIS ARG ASA

a surprisingly good condition while given a low-protein diet The blood ammonia levels were elevated but below the toxic border line. Smaller and more frequent meals further reduce the risks of postprandial hyperammonemia (17 22 24 29 38) The low protein diet, however gives rise to serious difficulties in the long run since it interferes with growth Thus our patients did not gain in length and one patient got even more brittle hair and developed a more keratotic skin Hyperammonemia may however occur despite a low to normal protein intake during febrile illnesses and other diseases with risks of endogenous catabolism and cause acute intoxication which can be illustrated as follows

When case no 3 was admitted to hospital at the age of 7.5 years for control we had the opportunity to observe the acute effect



Fig 4 Case no 1 (*a*) at the age of 10 years. (*b*) at the age of 11.5 years after 15 months on a low protein diet (0 3 g protein per kg bodyweight and day).

Fig 3 Effect on the plasma aminogram of different protein intake (*a*) Case no 1 (*b*) Case no 2. (*c*) Case no 3

c

Case no 3

Protein intake

(after ... follow up)

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Fig 3 Effect on the plasma aminogram of different protein intake (*a*) Case no 1 (*b*) Case no. 2. (*c*) Case no 3

c

Case no 3

Protein intake 0.5g per kg bodyweight
0.2
2.5g
0.3 (after 15 months follow up)

TAU THR SER GLU-NH2 PRO GLU CIT GLY ALA VAL CYS MET ILEU LEU TYR PHE ORN LYS HIS ARG ASA

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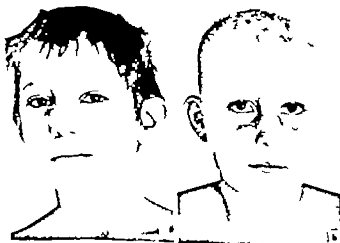


Fig 4 Case no 1 (*a*) at the age of 10 years, (*b*) at the age of 11.5 years after 15 months on a low protein diet (0.3 g protein per kg bodyweight and day)

of fever vomiting and diarrhoea caused by a viral infection Very rapidly severe hyper ammonemia (425 μg/100 ml) and intoxication signs i e ataxia deterioration developed implicating a period of parenteral carbohydrate administration Within 2 days the blood ammonia levels decreased to 270 μg/100 ml and the intoxication signs yielded slowly This observation indicates that fever should be prevented in patients with ASA in order to minimize the risk of hyperammonemia due to endogenous catabolism

Since ASA is supposed to be due to an enzymatic defect in the urea cycle (19 20 36) the increased plasma levels of citrulline and AS when the patients were given a high protein diet would be predicted as well as the absence of alterations in the plasma level of arginine and ornithine More interesting however is that despite the supposed defect in the urea cycle our patients did produce urea and that the urea level in blood as well as the urea excretion in the urine was influenced by the protein intake (Fig 2 *a–c*) although the response was slower when compared with that of normals This finding might indicate that the block in the activity of argininosuccinase is not complete or since the alteration in urea concentration does not seem to be paralleled by an alteration in the arginine or ornithine levels that there might be an alternative extrahepatic production of urea

Another interesting finding in our patients is the lack of increase in the plasma alanine levels in parallel to the hyperammonemia secondary to the high protein intake A specific elevation of plasma alanine levels have been reported in some other diseases i e familial protein intolerance (26) citrullinemia (28) hyperornithinemia and homocitrullinemia (33) where there is a hyper ammonemia due to abnormalities in the urea cycle enzymes Alanine has been supposed to play an essential role in the transport of amino groups through the glucose-alanine cycle (13) and the binding of amino groups to pyruvate to form alanine seems to represent a nontoxic alternative to ammonia for the transport of amino groups from the muscle to liver Furthermore Brosnan and his collaborators (6) has shown that alanine serves as an intrahepatic ammonia-binding agent in rats when urea synthesis ceases in anoxic liver In our patients the alanine levels were however most increased during the low protein diet period when the plasma ammonia levels were within the normal limits This finding seems to be in agreement with the findings in patients with kwashiorkor (4 34) or in normal children given a low protein diet (16) This increase has been proposed to be due to a minimum need for gluconeogenesis (25)

During the high protein provocation an increase could also be observed in the plasma levels of the branched chain amino acids valine isoleucine and leucine as well as of proline tyrosine and lysine This was most pronounced in case no 3 Increased levels of proline and tyrosine have been observed in hepatic failure and may be secondary to a limited metabolic capacity of the liver The branched chain amino acids have been observed to reflect insulin release and be increased when there is a reduced muscle protein synthesis secondary to an impaired insulin secretion (12) The increased plasma levels of lysine and of the branched chain amino acids during the high protein intake period may reflect a limited muscle protein synthetic capacity probably secondary to the ammonia intoxication

ACKNOWLEDGEMENT

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METABOLIC ALKALOSIS IN OBSTRUCTIVE VOMITING

Volume Depletion and Balance of Net Acid

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ABSTRACT Degn, J. K., Wamberg, S., Engel, K. and Kildeberg, P. (Departments of Paediatrics, Clinical Chemistry and Physiology Odense University Odense, Denmark) Metabolic alkalosis in obstructive vomiting. Volume depletion and balance of net acid. Acta Paediatr Scand, 63: 537 1974.—Pre-operative balances of sodium, potassium, calcium, magnesium, chloride and phosphorus were determined in eleven infants with hypertrophic pyloric stenosis. Additional measurements included acid-base status of blood and urine and concentrations of electrolytes in serum. From the balance data, the rate of retention of net acid (NAB), the pre-operative change in chloride space (ΔECW), and the effect of extracellular volume expansion on blood "base excess" (BE) were derived. The observed pre-operative change in blood BE was partitioned in retentional, dilutional, and distributional components by comparing estimates of such components based on known physiological constants with the results of multiple correlation analysis involving five predictor variables. The results indicate that the fall in blood BE to the normal range was almost entirely accounted for by volume expansion, and that retained net acid was probably neutralized by skeletal base.

KEY WORDS: Metabolic alkalosis, contraction alkalosis, acid-base metabolism, pyloric stenosis

Clinical disturbances of acid–base metabolism may be categorized according to primary and secondary deviations in concentrations in blood of three kinds of acid (or base) (1) carbonic acid (2) metabolizable organic acid and base (3) non-carbonic non-metabolizable acid and base By definition the term *net acid* refers to *titratable quantities* of acid and base belonging to the latter category (31) cf p 540 Metabolic alkalosis may be conceived as a disturbance in which the acid–base status of blood is primarily changed by a decrease in the concentration of non-carbonic acid On logical grounds such concentration changes may originate in any one of three independent ways viz. a net gain or net loss of solute, a redistribution of solute between compartments or a change in solvent volume.

Disregarding the possibility of significant changes in concentrations in blood of metabolizable organic acid and base including ionic species (1 5 6 17 22, 51) changes in the concentration of non-carbonic titratable acid (ΔBE_{blood}) will equal changes in the concentration of net acid (ΔNA_{blood}) Also in the case of iso-osmolar dehydration with exclusive depletion of the extracellular volume changes in the concentration of water in blood will be due solely to loss of water from the body Under these conditions therefore the overall change in blood BE is determined by the *balance of net acid*

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ABSTRACT Degn, J K., Wamberg, S., Engel, K. and Kildeberg, P (Departments of Paediatrics, Clinical Chemistry and Physiology Odense University Odense, Denmark). Metabolic alkalosis in obstructive vomiting. Volume depletion and balance of net acid. Acta Paediatr Scand, 63: 537 1974.—Pre-operative balances of sodium, potassium, calcium, magnesium, chloride, and phosphorus were determined in eleven infants with hypertrophic pyloric stenosis. Additional measurements included acid-base status of blood and urine and concentrations of electrolytes in serum. From the balance data, the rate of retention of net acid (NAB), the pre-operative change in chloride space (ΔECW), and the effect of extracellular volume expansion on blood "base excess" (BE) were derived. The observed pre-operative change in blood BE was partitioned in retentional, dilutional, and distributional components by comparing estimates of such components based on known physiological constants with the results of multiple correlation analysis involving five predictor variables. The results indicate that the fall in blood BE to the normal range was almost entirely accounted for by volume expansion, and that retained net acid was probably neutralized by skeletal base.

KEY WORDS: Metabolic alkalosis, contraction alkalosis, acid-base metabolism, pyloric stenosis

Clinical disturbances of acid–base metabolism may be categorized according to primary and secondary deviations in concentrations in blood of three kinds of acid (or base): (1) carbonic acid (2) metabolizable organic acid and base (3) non-carbonic non-metabolizable acid and base By definition the term *net acid* refers to *titratable quantities* of acid and base belonging to the latter category (31) cf p 540. Metabolic alkalosis may be conceived as a disturbance in which the acid–base status of blood is primarily changed by a decrease in the concentration of non-carbonic acid. On logical grounds, such concentration changes may originate in any one of three independent ways viz. a net gain or net loss of solute a redistribution of solute between compartments or a change in solvent volume

Disregarding the possibility of significant changes in concentrations in blood of metabolizable organic acid and base including ionic species (1 5 6 17 22 51) changes in the concentration of non-carbonic titratable acid (ΔBE_{blood}) will equal changes in the concentration of net acid (ΔNA_{blood}) Also in the case of iso-osmolar dehydration with exclusive depletion of the extracellular volume changes in the concentration of water in blood will be due solely to loss of water from the body Under these conditions therefore the overall change in blood BE is determined by the *balance of net acid*

(NAB) the *balance* of water and the (internal) *distribution of hydrogen ion* at any level of plasma P_{CO_2} and the change in blood BE may be evaluated statistically in terms of predictors of these three variables

Review of Problem

The effects of selective depletion of (gastric) hydrochloric acid in man have been described by Kassirer & Schwartz (25) and the acid–base response to isolated contraction by Cannon et al (10) However studies on the relative importance of these variables in the production of clinical gastric alkalosis apparently do not exist

Hydrochloric acid depletion and balance of net acid

In metabolic alkalosis due to vomiting the primary rise in blood BE is generally conceived as an immediate chemical consequence of removal of gastric hydrochloric acid from the body (28 35 36 47 48) and treatment with ammonium chloride is based on that assumption (27) However theoretical arguments may be advanced against the hypothesis of simple hydrochloric acid depletion (30) Thus losses of hydrogen ion from the stomach do not necessarily imply losses of hydrogen ion from the body since variables of the NAB include the rate of fecal net acid excretion the rate of endogenous production of hydrogen ion and the renal response Also it has been pointed out that the blood acid–base chemical effect of gastric transport of hydrogen ion is manifest at the moment of secretion and essentially independent of the subsequent fate of luminal acid (30) and evidence has been adduced in the healthy premature infant of a regulated rate of intestinal NA absorption within limits of oral NA intake (31 29) Variations in the rate of fecal NA excretion and in the size of the transcellular NA pool might influence materially the relation of depletion of gastric hydrochloric acid to the blood BE and discontinuation of oral feedings might alter the rate of endogenous production of NA and the rate of renal losses of filtered organic anion Finally the possibility remains that in obstructive vomiting a significant NA deficit may develop which is distributed largely on non-vascular buffer systems

Volume depletion

The *obligatory* effect of volume contraction is due to the fact that the titratable acidity of water on titration to pH 7 40 at a P_{CO_2} of 40 mmHg (i e the BE of water) is −25 5 mEq/l In terms of an end point at pH 7 40 and P_{CO_2} *zero* mmHg the effect of contraction is explained by variations in the amount of (neutral) solvent available for dissolution of base in blood On contraction of blood by removal of plasma water the change in blood BE is given by the expression

$$\Delta BE = -\frac{N}{N+1}\ (25\ 5 + BE_{initial}) \qquad (i)$$

where N is the (positive or negative) number of parts of water added to one part of blood i e the ratio of change in blood volume to initial blood volume Eq (i) states that both preformed base (of normal blood) and any additional quantity (mEq/l) are subject to concentration (30)

Hydrogen ion distribution

Changes in the internal distribution of net acid must be considered in the interpretation of the observed change in blood BE With the development of compensatory hypercapnia (26) an internal re-arrangement of bicarbonate occurs in the course of *in vivo* CO_2 titration (7 12 18 40) As a result a decrement in BE_{blood} originates the *magnitude* of which is determined not only by the level of P_{CO_2} but also by body composition (15) Also cellular losses of potassium might to some extent occur in exchange for extracellular hydrogen ion (13 38)

Formulation of Problem

cording to the foregoing discussion the al rise in blood BE may be partitioned in *entional contractional* and *distributional* rements

$$\Delta BE_{observed} = \Delta BE_{retention} + \Delta BE_{contraction} + \Delta BE_{distribution} \quad \text{(ii)}$$

may be pointed out that the fact that the velopment of any type of alkalosis re nires maintenance or "continuous re-roduction by an elevation of the rate of nal tubular conservation of base is of no onsequence to interpretation along these nes

Introducing five predictor variables— $\Delta BE_{retention} = F(NAB)$ $\Delta BE_{contraction} = F(\Delta ECW$ $BE_{initial})$ where ΔECW is the extracellular volume deficit and $\Delta BE_{distribution} = F(\Delta P_{CO_2},$ K loss)—we get a working equation (and five partial derivatives)·

$$\Delta BE_{observed} = F(NAB \;\; \Delta ECW \;\; BE_{initial} \;\; \Delta P_{CO_2} \;\; K \;\; loss) \quad \text{(iii)}$$

From an experimental point of view eq (iii) may be approached in two different ways (1) *Multiple correlation analysis* will provide a system of linear regression coeffi cients suggesting relative orders of magnitude for the composite parameters of eq (iii) as well as the relative significance of the predictors of that equation. (2) The partial derivatives may also be estimated by *direct calculation* based on knowledge of the appropriate physiological constants Comparison of such statistical and physiological estimates should be helpful in deciding upon the relevancy of the predictors under consideration The present study views the metabolic alkalosis of vomiting as the mirror image of its pre-operative reversal Some limitations of that approach are discussed on p 547

CASE MATERIAL

Eleven infants with hypertrophic pyloric stenosis were studied during the pre-operative period lasting from 31 to 91 hours (average 53 hours) The patients were admitted consecutively during the period October 1st, 1971 to March 16th, 1972. Pertinent clinical data are summarized in Table 1 When the diagnosis of pyloric stenosis was established collection of stool and urine was started, and blood was drawn for acid–base and

Table 1 *Clinical data of eleven infants with hypertrophic pyloric stenosis*

Patient no	Birth weight (kg)	Age (weeks)	Weight on admission (kg)	Period of pre-operative study (hours)	Blood acid–base status and serum electrolyte and urea concentrations on admission*							
					Na	K	Cl^-	pH	P_{CO_2}	BE	urea	ΔP_{CO_2}
I	3 90	4	3 97	72	138	4.8	88	7.52	47	+13.0		−7
II	3.20	5	3 46	48	145	5.0	97	7.53	40	+ 9.2	5.0	10
III	3 50	4	3.36	39	137	5 1	94	7.61	30	+ 8.5		10
IV	2 85	6	2 77	71	128	5 4	97	7 48	40	+ 6 0	7.6	3
V	3 60	7	4.84	39	134	4.3	95	7.58	38	+13.0		2
VI	2.65	6	3 37	63	135	3.5	77	7.65	37	+17.8	7.8	−1
VII	2 20	4	71	35	141	4.9	94	7.64	31	+11.8	4.3	2
VIII	3 10	5	3 50	47	140	5 9	96	7.54	35	+ 6.8	7.6	5
IX	3.30	4	3.50	91	143	5.0	99	7 41	48	+ 4.9	4 4	−12
X	3 70	4	4 10	41	140	4 4	96	7.65	31	+11.8		7
XI	3.33	6	4.40	41	142	5.4	99	7.50	36	+ 4.6	3.2	5
Mean	3.23	5	3.63	53	138	4 9	94	7.56	38	+ 9.8		2

Patient no. IV had previously been operated for a lumbar myelomeningocele and associated hydrocephalus. At the time of admission for pyloric stenosis, a ventriculo-caval shunt was functioning satisfactorily Patient no. IV was born at term with signs of moderate intra-uterine growth retardation no neonatal problems. Patient VII was born 10 days after expected date of confinement as the first of (discordant) twins, no neonatal problems.

* Sodium, potassium, chloride and urea concentrations are in mmoles/l P_{CO_2} is in mmHg; BE is in mEq/l. ΔP_{CO_2} is the observed pre-operative change in P_{CO_2}.

electrolyte studies. In most cases a parenteral regimen was substituted for oral feedings soon after admission In each patient a pyloric tumour was found at operation and each made an uneventful recovery

METHODS

Sample preparation

Urine was collected quantitatively by the method of Liu & Anderson (34) as modified by Clifton (11) *Stool* and *vomited material* were collected quantitatively in minimal amounts of electrolyte-free paper diaper In some patients vomiting was avoided by continual gastric suction the aspirate being stored for analysis. Volumes of *parenteral fluids* and of any *oral preparations* given were registered carefully and samples were stored for analysis Storage took place in deep-freezer at −25°C Total pre-operative stool specimens were thawed and dried at 105°C overnight After adding a few ml of alcohol the material was ignited and burned in a Pyrex beaker in a gentle air stream to remove the volume of paper The beaker was subsequently rinsed with 0 7 N nitric acid and the contents transferred to a one-litre round flask fitted in an electric heater and boiled for 1 hour with a reflux mount After cooling and centrifuging aliquots were obtained for analysis. Vomited material was extracted with distilled water for 2 hours at 100°C in a rotating mixer

Chemical methods

Concentrations of *sodium* and *potassium* were measured by microprocedures using the Instrumentation Laboratories model 343 flame photometer and microdetermination of concentrations of *calcium* and *magnesium* were carried out by means of the Perkin-Elmer model 403 atomic absorption spectrophotometer Concentrations of *chloride* and (total) *phosphorus* were measured by the methods of Cotlove et al. (14) and Baginski (3 4 23) respectively *Urinary titratable acid ammonium* and *titratable organic anion* were measured by potentiometric titration at 37°C using the Radiometer TTT2 titrator with autoburette ABU 13 (?1 24 31 45) Concentrations of *sulphate* in urine were determined by the method of Yatzidis et al. (50) and the *acid–base status of* (capillary) *blood* by the equilibration technique (42)

Calculations

Balance of net acid

Non-carbonic acids as well as bases may be either anionic cationic or non-ionic and each of these may be either metabolizable or non-metabolizable Defining the *net acid concentration* of any buffer solution containing representatives of some or all of these twelve categories as the titratable acidity of that solution on titration to an end-point at pH 7 40 temperature 37°C ionic strength of normal plasma, P_{CO_2} zero mmHg, and concentration of (non-ionic) metabolizable acid and base equal to zero—the principle of electroneutrality offers the simplest basis for its derivation (31 33 41). Thus,

$$NAC = (\text{anion})_{nm} - (\text{cation})_{nm} \qquad \text{(iv)}$$

where NAC is the net acid concentration and ( ) denote equivalent concentrations *at the end-point* The subscript "nm identifies non-metabolizable species. Eq (iv) may be applied to diet and stool as follows:

$$NA = [Cl^-] + 1.8[P] - [Na^+] - [K^+] - 2[Ca^{++}] - 2[Mg^{++}] \qquad \text{(v)}$$

where [ ] denote rates of intake or loss in mmoles per period of observation For urine sulfate ions must be considered as well However taking the rate of renal sulphate excretion to match the rate of endogenous production of sulphuric acid (zero sulphate balance) sulphate may be disregarded and the *balance* of net acid derived as

$$NAB = (B_{Cl^-} + 1.8\,B_P) - (B_{Na^+} + B_{K^+} + 2\,B_{Ca^{++}} + 2\,B_{Mg^{++}}) \qquad \text{(vi)}$$

where B are molar balances representing oral and parenteral intake and gastrointestinal and renal loss

The NAB may also be assessed by direct titration (9 44) However because of errors inherent in titration procedures, differences between (the extent of) *in vitro* and *in vivo* oxidation of nutrients, particularly in the growing infant as well as of several technical aspects *we find evaluation according to eq (vi) superior* Also contrary to titration methods application of eq (vi) permits of distinction between subtraction disturbances and "addition disturbances"

In order to properly *interpret* the renal net acid excretion as evaluated according to eq (v) urinary ammonium, titratable acidity titratable organic anion and sulphate must be measured as well. For direct evaluation renal net acid excretion may be expressed as

$$NAE = NH_4^+ + TA - [OA^-] \qquad \text{(vii)}$$

where TA is the titratable acidity on titration of urine to pH 7 40 and P_{CO_2} *zero* mmHg and $[OA^-]$ is urinary titratable organic anion In our experience results obtained according to eqs (iv) and (vii) agree well (Table 3)

Change in plasma volume

The actual (initial) extracellular water volume (ECW_a) was estimated as

$$ECW = \frac{33.6264 - 0.8928\,B_{Cl}}{[Cl^-]_a} \quad (\text{l/kg}) \qquad \text{(viii)}$$

which implies a Donnan factor of 0.96, a normal ECW of 0 320 l/kg body weight (20) a concentration of water in serum of 0 93 kg/l and a normal concentration of chloride in serum $[Cl^-_n]$, of 100 mmols/l Furthermore,

$$\Delta ECW = 0.320 - ECW \quad (\text{l/kg}) \qquad \text{(ix)}$$

Assuming the normal volume of plasma water to be 0 93×0.050=0 047 l/kg body weight, the extracellular

volume deficit was distributed proportionately on the intra- and extravascular phases by taking (0.047×100/0.320=)14.69% of the ΔECW to represent the change in blood volume (ΔBV) incident to loss of that amount of plasma water

Expansion effect

Finally an estimate of the "true" effect of pre operative ECW expansion (ΔBE_{expan}) was obtained by inserting

$$N = \frac{\Delta BV}{0.080 - BV} \qquad (x)$$

and the initial blood BE in eq (i)

Multiple linear regression analysis

Multiple regression analysis involving two to five variables was carried out by means of the Hewlett-Packard 9810A calculator and according to the principles described by Fisher (19) and by Armitage (2) The statistical symbols used are defined in the legend of Table 5

RESULTS

Components of the average pre-operative balances of sodium, potassium calcium magnesium, chloride and phosphorus as well as of the resulting pre-operative balances of net acid are presented in Table 2. As expected these infants retained sodium and potassium at roughly equimolar rates and chloride at a rate exceeding the rate of retention of mineral cation The potassium balances probably do not reflect pre-admission losses because complete replenishment of body stores of potassium could hardly occur within the relatively short period of observation. The finding of significant potassium retention despite slightly negative balances of phosphorus (zero intake) may indicate that potassium had been lost in part by ionic exchange across the cell membrane Pre-operative balances of calcium and magnesium were close to zero while rates of retention of net acid in individual patients ranged from −1 1 to +22.1 mEq/kg body weight Interestingly the pre-operative NAB was negatively and significantly correlated to the balances of calcium and magnesium The following relationship was found

$$NAB = -0.22 - 7.47\, B_{Ca^{++}} - 4.54\, B_{Mg^{++}} \qquad (xi)$$

$(R = 0.83 \;\; P < 0.01)$

The average balances of calcium and net acid (Table 2) suggest that about 15% of the pre-admission accumulation of net base had been deposited in the skeleton as apatite (28)

Supplementary urinary results are given in Table 3 The data document the phenom-

Table 2. *Average pre-operative balances of sodium potassium calcium magnesium chloride phosphorus and net acid in eleven infants with hypertrophic pyloric stenosis*

	Diet	Stool	Vomit & aspirate	Absorption	Urine	Parenteral intake	Balance
Sodium	0.034 (0.113)	0.239 (0.221)	3.196 (3.288)	−3.400 (3.301)	2.233 (2.289)	7.893 (4.094)	2.260 (2.041)
Potassium	0.061 (0.[illegible]03)	0.357 (0.296)	0.869 (1.028)	−1.165 (1.17[illegible])	2.548 (1.668)	5.650 (3.153)	1.937 (1.588)
Calcium	0.026 (0.087)	0.521 (0.689)	0.115 (0.118)	−0.610 (0.644)	0.165 (0.120)	0.001 (0.003)	−0.773 (0.674)
Magnesium	0.006 (0.020)	0.227 (0.315)	0.051 (0.041)	−0.272 (0.304)	0.157 (0.080)	0.253 (0.201)	−0.176 (0.400)
Chloride	0.037 (0.122)	0.114 (0.162)	5.371 (6.813)	−5.448 (6.833)	1.396 (2.461)	15.609 (10.003)	8.766 (8.119)
Phosphorus	0.032 (0.105)	0.314 (0.368)	0.151 (0.171)	−0.434 (0.345)	0.623 (0.289)	0.008 (0.022)	−1.049 (0.487)
Net acid	−0.066 (0.[illegible]19)	−1.414 (1.408)	1.[illegible]47 (2.918)	0.101 (3.241)	−2.070* (2.083)	1.572 (5.510)	4.579 (6.881)

Values stated are in mmoles per kg body weight per period of observation except that net acid is in mEq Figures in parentheses are standard deviations.

* Sulphate loss included, cf p. 540

Table 3 *Renal excretion of acid in eleven infants with hypertrophic pyloric stenosis*

Patient no	$NA_{(i)}$	$NA_{(ii)}$	$TA+NH_4$	OA	$SO_4^=$
I	−5 872	−5 63[illegible]	2 975	8 607	0.514
II	−1.291	−1 445	1 31[illegible]	2 757	0.387
III	−1 306	−1.224	0.397	1 621	0.276
IV	−4 481	−5 939	1 14[illegible]	7 081	0 406
V	−0.570	−0 5[illegible]0	1 132	1 652	0.338
VI	−3 151	−3 347	0 719	4 066	0.201
VII	−1.381	−1 387	0 431	1.818	0.301
VIII	−0 151	−0 022	1.53[illegible]	1 554	0 188
IX	−2.303	−3 802	1 626	5 428	1.206
X	−0 149	−0.804	0 728	1.53[illegible]	0 09[illegible]
XI	−[illegible] 117	−2 627	−0 084	2.543	0 692
Mean	−2 070	−[illegible] 43[illegible]	1 083	3.514	0 418

$NA_{(i)}$ Urinary net acid according to eq (iv) sulphate being included as a non-metabolizable anion
$NA_{(ii)}$ Urinary net acid according to eq (vii)
TA Urinary titratable acid on titration to pH 7 40 P_{CO_2} zero mmHg, and temperature 37°C
OA Urinary titratable organic acid on titration from pH 2.70 to pH 7 40 at 37°C

Values stated are in mEq per kg body weight per period of observation (Table 1) except that sulphate excretion is in mmoles

$NA_{(ii)} = -0.22 + 1\ 06\ NA_{(i)}$ by linear regression analysis ($r=0\ 95$ $p<0\ 001$)

enon of paradoxical aciduria (46) in that the sum of urinary ammonium ion and titratable acid was positive in all patients except one The rate of excretion of $NH_4^+ + TA$ (<0 5 mEq/kg/24 hours) however was clearly lower than normal and the urinary net acid values (first and second colum actually show a loss of *net base* from t body at an average rate of about one mE kg/24 hours The rate of excretion of titr table organic anion was within normal limi for infants of this age group[1]—supportin the assumption (p 537) that major change in the concentration in blood of metaboliz able organic acid and base did not occur The rate of excretion of sulphate was low

The estimated change in chloride space and the corresponding pre-operative expansion effect are shown in Table 4 The figures indicate a pre admission reduction in extracellular fluid volume ranging from less than 10 to about 170 ml per kg admission weight Because the sodium concentrations were largely normal (Table 1) these values represent an almost identical loss of body water Thus in the most severely dehydrated patient (no VI) the water deficit amounted to 14–15% of the estimated normal (rehydrated) body weight

Results of statistical evaluation based upon data on single patients are presented in Table 5 It is seen that the $\Delta BE_{observed}$ is negatively correlated to the NAB at the

[1] 1 42±0.51 mEq/kg/24 hours according to preliminary data from this laboratory

Table 4 *Eleven infants with hypertrophic pyloric stenosis Calculated depletion of extracellular volume (chloride space) calculated pre-operative expansion effect and observed pre-operative change in blood BE*

Patient no	ECW_r (l/kg)	ΔECW (l/kg)	$\Delta BE_{exp\ in}$ (mEq/l)	$\Delta BE_{exp\ ex}$ (mEq/l)	ΔBE_{obs} (mEq/l)
I	0 1676	0 1574	−10 775	−15 125	−13 2
II	0.3584	−0 0384	[illegible] 446	0 608	1 7
III	0 2920	0 0280	−1 747	−3 686	−0 7
IV	0 3158	0 0042	−0.[illegible]44	−0 540	4.8
V	0.3050	0 0150	−1 059	−5 393	−5 9
VI	0 1479	0 1721	−13 683	−19.324	−17 6
VII	0 2434	0 0766	−5 245	−9 056	−12 8
VIII	0.3171	0 0029	−0 174	−0 915	0 6
IX	0 2255	0 0945	−5.274	−6.[illegible]93	−7 9
X	0.3120	0 0080	−0.550	−4 197	−7 8
XI	0.3055	0 0145	−0 801	−0 452	−4 6
Mean	0.2718	0.0482	−3 373	−5 852	−5 8

$\Delta BE_{exp\ ex} = 3.261 - 70.830\ \Delta ECW - 0.584\ BE_{initial}$, cf p 544 $\Delta BE_{exp\ ex} = 1\ 03 + 0\ 75 \cdot \Delta BE_{exp\ in}$ ($r=0\ 97$ $P<0.001$)
$\Delta BE_{obs} = -1\ 68 + 1.21\ \Delta BE_{exp\ ex}$ ($r=0\ 86$ $p<0\ 001$)

Table 5 *Multiple linear regression analysis of variables of the change in blood be caused by obstructive vomiting*

The notation used conforms with the general expression: $Y = a + b_1x + b_2x + b\ x \quad b_nx_n$

Total sums of squares (SS_{total}) represent sums of squared deviations from the mean of the y distribution (ȳ); *residual sums of squares* ($SS_{residual}$) represent sums of squared deviations from the regression line (Y); *regression sums of squares* ($SS_{regression}$), differences between these two, represent the amount of variation accounted for by the regression function concerned

R = coefficient of multiple correlation $= \sqrt{\frac{SS_{regression}}{SS_{total}}}$ MS = mean square $= \frac{SS}{DF}$ $F = \frac{MS_{regression}}{MS_{residual}}$ $z = 0.5 \ln F$

Data on individual patients used for correlation analysis may be obtained from the authors (P. K.)

Analysis no.	Regression variables: y	x	x_2	x	x	Regression parameters: a	b	b_2	b_3	b
1	ΔBE[illegible]	NAB				−1.896	−0.845			
2	ΔBE[illegible]	ΔECW				−1.479	−88.951			
3	ΔBE[illegible]	BE[illegible]				5.961	−1.201			
4	ΔBE[illegible]	B_{K^+}				0.694	−3.334			
5	ΔBE[illegible]	ΔP_{CO_2}				−6.982	0.558			
6	ΔBE[illegible]	ΔECW	BE[illegible]			3.189	−69.455	−0.574		
7	ΔBE[illegible]	NAB	ΔECW	BE[illegible]		3.261	0.019	−70.830	−0.584	
8	ΔBE[illegible]	ΔECW	BE[illegible]	B_{K^+}		3.439	−64.450	−0.579	−0.230	
9	ΔBE[illegible]	NAB	ΔECW	BE[illegible]	B_K	3.397	0.049	−58.041	−0.557	−0.362
10	ΔBE[illegible]	NAB	ΔECW	BE[illegible]	ΔP_{CO_2}	3.272	−0.003	−57.321	−0.667	0.111
11	ΔBE[illegible]	ΔBE[illegible]				−1.678	1.211			
12	NAB	B_{K^+}				−0.715	2.733			
13	NAB	$B_{Ca^{++}}$	$B_{Mg^{++}}$			−0.221	−7.468	−4.538		
14	NAB	ΔECW				0.300	88.846			

Analysis no	Simple (r) or multiple (R) correlation coefficient	Sums of squares: Total	Regression	Residual	Degrees of freedom: Total	Regression	Residual
1	−0.818 (r)	488.425	327.109	161.316			9
2	−0.853 (r)	488.425	355.330	133.095			9
3	−0.706 (r)	488.425	243.701	244.774			9
4	−0.758 (r)	488.425	280.292	208.133			9
5	0.540 (r)	488.425	142.677	345.748			9
6	0.898 (R)	488.425	394.006	94.419	10	2	8
7	0.898 (R)	488.425	394.033	94.392	10	3	7
8	0.898 (R)	488.425	394.280	94.145	10	3	7
9	0.899 (R)	488.425	394.368	94.057	10	4	6
10	0.900 (R)	488.425	395.958	92.467	10	4	6
11	0.857 (r)	488.425	358.856	129.569			9
12	0.641 (r)	458.472	188.432	270.040			9
13	0.831 (R)	458.472	316.820	141.652	10	2	8
14	0.879 (r)	458.472	354.490	103.982			9

Analysis no	Mean square: Total	Regression	Residual	Variance ratio (F)	z value	Probability (p)
1	48.843	327.109	17.974	18.250		0.001<P<0.010
2	48.843	355.330	14.788	24.028		P<0.001
3	48.843	243.701	27.192	8.96.		0.010<P<0,050
4	48.843	280.292	23.126	12.120		0.001<P<0.010
5	48.843	142.677	38.416	3.714		0.050<P<0.100
6	48.843	197.003	11.802	16.692	1.408	0.001<P<0.010
7	48.843	131.344	13.485	9.740	1.138	0.001<P<0.010
8	48.843	131.427	13.449	9.772	1.140	0.001<P<0.010
9	48.843	98.592	15.676	6.289	0.919	0.010<P<0.050
10	48.843	98.990	15.411	6.423	0.930	0.010<P<0.050
11	48.843	358.856	14.397	24.927		P<0.001
12	45.847	188.432	30.005	6.280		0.010<P<0.050
13	45.847	158.410	17.707	8.946	1.096	0.001<P<0.010
14	[illegible]	354.490	11.554	30.682		P<0.001

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II	−1.291	−1 445	1.31?	2.757	0 387
III	−1 306	−1 224	0.397	1 621	0.276
IV	−4 481	−5 939	1 14?	7 081	0 406
V	−0.570	−0 520	1 132	1 65?	0 338
VI	−3 151	−3 347	0 719	4 066	0 201
VII	−1.381	−1 387	0 431	1.818	0.301
VIII	−0 151	−0 022	1.532	1.554	0 188
IX	−2.303	−3.802	1.626	5 428	1 206
X	−0 149	−0 804	0 728	1.532	0 092
XI	−? 117	−? 627	−0 084	?.543	0 69?
Mean	−2.070	−? 432	1 083	3.514	0 418

$NA_{(i)}$ Urinary net acid according to eq (iv) sulphate being included as a non metabolizable anion
$NA_{(vii)}$ Urinary net acid according to eq (vii)
TA Urinary titratable acid on titration to pH 7 40 P_{CO_2} zero mmHg, and temperature 37°C
OA Urinary titratable organic acid on titration from pH 2 70 to pH 7 40 at 37°C

Values stated are in mEq per kg body weight per period of observation (Table 1) except that sulphate excretion is in mmoles

$NA_{(vii)} = -0.22 + 1\ 06\ NA_{(i)}$ by linear regression analysis ($r = 0\ 95$ $p < 0\ 001$)

enon of paradoxical aciduria (46) in that the sum of urinary ammonium ion and titratable acid was positive in all patients except one The rate of excretion of NH^+_4+TA (<0 5 mEq/kg/24 hours) however was clearly lower than normal and the urinary net acid values (first and second column actually show a loss of *net base* from t body at an average rate of about one mE kg/24 hours The rate of excretion of titr table organic anion was within normal limi for infants of this age group[1]—supportin the assumption (p 537) that major change in the concentration in blood of metaboliz able organic acid and base did not occur The rate of excretion of sulphate was low

The estimated change in chloride space and the corresponding pre-operative expansion effect are shown in Table 4 The figures indicate a pre admission reduction in extracellular fluid volume ranging from less than 10 to about 170 ml per kg admission weight Because the sodium concentrations were largely normal (Table 1) these values represent an almost identical loss of body water Thus in the most severely dehydrated patient (no VI) the water deficit amounted to 14–15% of the estimated normal (rehydrated) body weight

Results of statistical evaluation based upon data on single patients are presented in Table 5 It is seen that the $\Delta BE_{observed}$ is negatively correlated to the NAB at the

[1] 1 42±0.51 mEq/kg/24 hours according to preliminary data from this laboratory

Table 4 *Eleven infants with hypertrophic pyloric stenosis Calculated depletion of extracellular volume (chloride space) calculated pre-operative expansion effect and observed pre-operative change in blood BE*

Patient no	ECW_s (l/kg)	ΔECW (l/kg)	$\Delta BE_{exp\ b}$ (mEq/l)	$\Delta BE_{exp\ a}$ (mEq/l)	ΔBE_{obs} (mEq/l)
I	0 1676	0 1524	−10 775	−15 125	−13 ?
II	0.3584	−0 0384	2 446	0 608	1 7
III	0.2920	0 0280	−1 747	−3 686	−0 7
IV	0 3158	0 0042	−0.244	−0.540	4.8
V	0 3050	0 0150	−1 059	−5.393	−5 9
VI	0 1479	0 1721	−13 683	−19 324	−17 6
VII	0 2434	0 0766	−5.245	−9 056	−12.8
VIII	0 3171	0 0029	−0 174	−0 915	0 6
IX	0 2255	0 0945	−5 274	−6.?93	−7 9
X	0.3120	0 0080	−0 550	−4 197	−7 8
XI	0.3055	0 0145	−0 801	−0 452	−4 6
Mean	0 2718	0 0482	−3 373	−5 852	−5 8

$\Delta BE_{exp\ b} = 3.261 - 70.830\ \Delta ECW - 0.584\ BE_{normal}$ cf p 544 $\Delta BE_{exp\ a} = 1\ 03 + 0\ 75\ \Delta BE_{exp\ b}$ ($r = 0.97$ $P < 0.001$).
$\Delta BE_{obs} = -1\ 68 + 1.21\ \Delta BE_{exp\ a}$ ($r = 0.86$ $p < 0\ 001$)

Table 5 Multiple linear regression analysis of variables of the change in blood be caused by obstructive vomiting

The notation used conforms with the general expression: $Y = a + b\,x + b_2 x + b\,x \quad b_n x_n$

Total sums of squares (SS_{total}) represent sums of squared deviations from the mean of the y distribution ($\bar{y}$); *residual sums of squares* ($SS_{residual}$) represent sums of squared deviations from the regression line (Y); *regression sums of squares* ($SS_{regression}$), differences between these two represent the amount of variation accounted for by the regression function concerned.

R=coefficient of multiple correlation $= \sqrt{\dfrac{SS_{regression}}{SS_{total}}}$ MS=mean square $= \dfrac{SS}{DF}$ $F = \dfrac{MS_{regression}}{MS_{residual}}$ $z = 0.5 \ln F$

Data on individual patients used for correlation analysis may be obtained from the authors (P K)

Analysis no.	Regression variables					Regression parameters				
	y	x	x	x_3	x	a	b	b_2	b	b
1	ΔBE	NAB				−1 896	−0.845			
	ΔBE	ΔECW				−1 479	−88 951			
3	ΔBE	BE				5 961	−1.201			
4	ΔBE	B_{K^+}				0 694	−3.334			
5	ΔBE	ΔP_{CO_2}				−6.982	0.558			
6	ΔBE	ΔECW	BE			3 189	−69 455	−0.574		
7	ΔBE	NAB	ΔECW	BE		3.261	0.019	−70.830	−0.584	
8	ΔBE	ΔECW	BE	B_{K^+}		3 439	−64 490	−0.579	−0 230	
9	ΔBE	NAB	ΔECW	BE	B_{K^+}	3.397	0.049	−58 041	−0.557	−0 36..
10	ΔBE	NAB	ΔECW	BE	ΔP_{CO_2}	3.272	−0.003	−57.321	−0 667	0 118
11	ΔBE	ΔBE				−1 678	1.211			
12	NAB	B_{K^+}				−0 715	2.733			
13	NAB	$B_{Ca^{++}}$	$B_{Mg^{++}}$			−0.321	−7 468	−4.538		
14	NAB	ΔECW				0 300	88.846			

Analysis no.	Simple (r) or multiple (R) correlation coefficient	Sums of squares			Degrees of freedom		
		Total	Regression	Residual	Total	Regression	Residual
1	−0.818 (r)	488.425	327 109	161.316			9
	−0 853 (r)	488 425	355.330	133.095			9
3	−0.706 (r)	488 425	243 701	244 724			9
4	−0.758 (r)	488 425	280.292	208.133			9
5	0 540 (r)	488 425	142.677	345 748			9
6	0 898 (R)	488.425	394.006	94 419	10	2	8
7	0 898 (R)	488 425	394.033	94.392	10	3	7
8	0.898 (R)	488 425	394.280	94 145	10	3	7
9	0.899 (R)	488 425	394 368	94.057	10	4	6
10	0 900 (R)	488 425	395.958	92.467	10	4	6
11	0 857 (r)	488.425	358.856	129.569			9
12	0 641 (r)	458.472	188 432	270 040			9
13	0 831 (R)	458.472	316.820	141.652	10	2	8
14	0 879 (r)	458 472	354 490	103 982			9

Analyses no.	Mean square			Variance ratio (F)	z value	Probability (p)
	Total	Regression	Residual			
1	48 843	327 109	17 924	18.250		$0.001 < P < 0.010$
2	48 843	355.330	14 788	24.028		$P < 0.001$
3	48 843	243 701	27 192	8.962		$0.010 < P < 0.050$
4	48 843	280.292	23 126	12.120		$0.001 < P < 0.010$
5	48 843	142.677	38 416	3 714		$0.050 < P < 0.100$
6	48 843	197.003	11.802	16.692	1 408	$0.001 < P < 0.010$
7	48.843	131.344	13 485	9 740	1 138	$0.001 < P < 0.010$
8	48 843	131.427	13 449	9 772	1 140	$0.001 < P < 0.010$
9	48 843	98.592	15.676	6.289	0 919	$0.010 < P < 0.050$
10	48 843	98 990	15 411	6.423	0.930	$0.010 < P < 0.050$
11	48.843	358.856	14.397	24 927		$P < 0.001$
12	45.847	188.432	30.005	6.280		$0.010 < P < 0.050$
13	45 847	158.410	17 707	8.946	1.096	$0.001 < P < 0.010$
14	45 847	354 490	11.554	30 682		$P < 0.001$

0 01 probability level—with a regression coefficient indicating an apparent volume of distribution of retained acid of 1 18 l/kg body weight It is seen also (no 14) that statistically the NAB depends heavily upon the ΔECW and that the inclusion of additional predictor variables (nos 7 9 and 10) raises the amount of variation of $\Delta BE_{observed}$ (sum of squares) accounted for by the regression equation from 67 to 81%—at the same time almost eliminating the NAB from the statistical interpretation Thus analysis no 7 gives a b_1 of only 0 019 which corresponds to an apparent volume of distribution of 50 l/kg body weight—or in physiological terms to a retention of 50 mEq/kg body weight of net acid per mEq retained in one litre of blood It may be noted that even a coefficient of NAB corresponding to the generally accepted volume of distribution of about 0 5 l/kg (39 42 43 49) would suffice to account for not more than 40% of the mean observed change in blood BE

By contrast the coefficients of ΔECW and $BE_{initial}$ in analyses nos 6 7 8 9 and 10 closely resemble the physiological parameters represented by the calculated $\Delta BE_{exp(a)}$ Thus analysis no 7 generates a statistical estimate of the expansion effect ($\Delta BE_{exp(a)}$) cf Table 4 which is a close reproduction of the $\Delta BE_{exp(a)}$ (r = 0 97 $p<0\ 001$) Moreover the average $\Delta BE_{observed}$ and the average $\Delta BE_{exp(a)}$ are identical (Table 4)

In terms of statistical *significance* analyses nos 2 and 6 provide the best formulation of the observed change in blood BE In the extended functions (nos 7 8 9 and 10) the inherent loss of degrees of freedom prevents a concomitant improvement of statistical significance and nothing is gained by including ΔP_{CO_2} and the balance of potassium

INTERPRETATION

The statistical approach to eq (iii) provides a numerical explanation of the relationship of the observed change in blood BE to the predictor variables selected for study Although combinations of four predictors (Table 5 nos 9 and 10) are more powerful in generating such explanation in that they provide the minimum residual variance they do not raise the level of statistical significance Several predictors are significantly correlated to the $\Delta BE_{observed}$ and moreover statistically interrelated to a considerable extent For example the change in chloride space and the NAB are interdependent partly because in the vomiting patient losses of water and losses of hydrochloric acid tend to vary in parallel and partly because under such circumstances both ΔECW and NAB may be derived from the balance of chloride with a fair degree of accuracy Obviously additional evidence is required in order to develop a causal argument

The ΔP_{CO_2} influences the $\Delta BE_{observed}$ to a small but predictable extent (15) and represents no obstacle to the physiological interpretation Clearly however the statistical recognition of this influence is

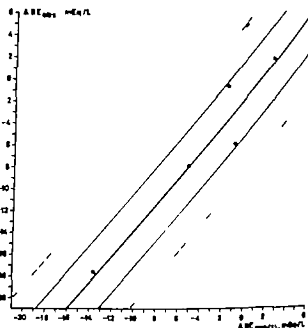


Fig 1 Relationship of observed pre-operative change in blood BE (ΔBE_{obs}) to the calculated expansion effect of pre-operative rehydration (cf Table 4). Distances are standard deviations from the regression line

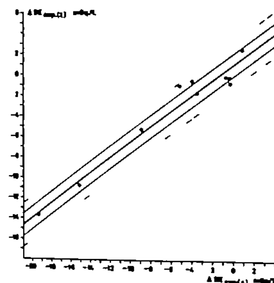


Fig. 2. Relationship of expansion effect of pre-operative rehydration calculated from known physiological constants (ΔBE_{expan}) to the corresponding estimate based upon multiple linear regression analysis (cf. Tables 4 and 5). Distances are standard deviations from the regression line.

compromised by the reciprocal relationship between BE_{blood} and P_{CO_2}. As far as the loss of potassium is concerned the present results lend no support to the concept of H/K exchange across the cell membrane as a major factor in the production of gastric alkalosis and neither do those of other authors (16, 37). In the present context we shall pursue the distributional component no further. On the other hand, in view of traditional concepts of gastric alkalosis, the relation of the NAB to water depletion in the causation of that disturbance appears particularly intriguing.

The patients studied by us did retain acid at a rate which was significantly correlated to the concomitant decrease in blood BE. Nevertheless, the analysis of the data obtained indicate that the $\Delta BE_{observed}$ was almost entirely due to expansion of the volume of solvent. The validity of this interpretation rests on the following points:

(1) The expansion effect is obligatory, i.e. a chemical necessity. Whereas it is not possible to change the extracellular water volume without influencing the concentration of net acid, it is theoretically possible for retained net acid to be deposited largely in non-extracellular compartments.

(2) Although the accuracy of the calculated expansion effect (ΔBE_{expan}) is limited by several assumptions (pp. 540–541) its order of magnitude cannot be disputed. In the vomiting patient, losses of 0.050 to 0.150 l/kg body weight of bicarbonate-free fluid influences the BE of blood to a degree which must be roughly similar to the (total) rise in BE commonly observed in such patients (30). Fig. 1 shows that the $\Delta BE_{observed}$ and the ΔBE_{expan} were very similar (regression coefficient 1.21).

(3) Whereas the coefficient of NAB found by statistical evaluation (Table 5, nos. 7, 9 and 10) is very different from that expected from previous experimental and clinical results (39, 42, 43, 49), the statistical parameters of the expansion effect are strikingly similar to the underlying physiological constants (Fig. 2).

(4) The observed change in the BE of blood correlates more tightly to the change in chloride space than to the balance of net acid (Table 5, nos. 1 and 2).

(5) A final point relates to the question of the location of retained net acid in the body. Table 6 shows, for each patient, the calculated amount of sodium lost from the extracellular fluid (ΔNa^+_{ECW}) during the pre-admission period of vomiting. Comparing the ΔNa^+_{ECW} with the sodium balances shows that an appreciable redistribution of body sodium must have occurred in addition to external losses. However, even assuming a mole to mole loss of extracellular sodium to the cell water in exchange for potassium lost from the body fails to account for the residual extracellular deficit. Apparently extracellular sodium, in amounts corresponding to at least $\Delta Na^+_{ECW} - B_N + -B_K$, had been deposited elsewhere in the body. These amounts were closely ($r = 0.94$) and signifi-

Table 6. *Eleven infants with hypertrophic pyloric stenosis: partition of losses of sodium from the extracellular fluid (chloride space)*

Patient no.	ΔNa_{ECW} (mmoles/kg)	B_{Na} (mmoles/kg)	B_K (mmoles/kg)	$\Delta Na_{ECW} - B_{Na} - B_K$ (mmoles/kg)	NAB (mEq/kg)
I	21.671	4.801	5.556	11.314	9.891
II	−7.168	−0.728	0.479	−6.919	−1.087
III	4.796	3.712	2.372	−1.288	−0.198
IV	4.378	0.950	0.331	3.097	0.395
V	3.930	2.943	1.082	−0.095	0.711
VI	24.834	2.918	3.466	18.450	22.100
VII	10.481	3.841	2.383	4.257	5.525
VIII	0.406	0.298	0.370	−0.217	2.461
IX	12.554	4.749	2.638	5.167	6.322
X	1.120	−0.739	1.071	0.788	4.988
XI	1.419	2.117	1.610	−2.308	−0.235
Mean	7.129	2.260	1.937	2.932	4.579

$\Delta Na_{ECW} = 0.370 \times 140 - [Na^+]_s \times ECW_s$; for $[Na^+]_s$ see Table 1.
$NAB = 1.906 + 0.912 (\Delta Na_{ECW} - B_{Na} - B_K)$; $r = 0.94$; $p < 0.001$.

cantly ($p<0.001$) correlated to the NAB (Table 6). The two right hand columns of the table suggest a one to one relationship between mmoles of extracellular sodium lost to non-soft tissues and mEqs of net base accumulated during the period of vomiting (regression coefficient 0.912). Deposition of sodium carbonate in the skeleton is an attractive possibility. Actually recent studies in dogs have documented carbonate deposition in tibial cortical bone in experimental alkalosis (8).

The diet of infants and children contains appreciable quantities of net base (29, 31, 32, 44) and throughout the phase of active body growth there is a continuous flux of net base across the gastrointestinal membrane to the developing tissues, in particular bone (28, 31). In the fasting and vomiting infant there is also a flux of net base to the portal blood. There are no obvious reasons why the vomiting infant should not deposit this absorbed base in the skeleton in the absence of calcium as sodium carbonate or equivalent—and escape hypernatremia and excessive increases in blood pH. The average pre-operative NAB in our patients was 2.23 mEq per kg and 24 hours—a figure which is quite comparable to the average daily base retention in normal growing infants (approx. 1.38 mEq/kg/24 h). It may be added that by the present balance technique the gastrointestinal lumen is included in the body and that theoretically part of the retained base may be present in an augmented transcellular pool in the lower intestinal tract (30).

The interpretation of recovery data in terms of pathogenetic mechanisms is a usual procedure with well known limitations. In the present case it may be argued that a primary retention alkalosis (HCl loss) may be temporarily compensated by dilution just as a primary contractional increment in blood BE may be alleviated by administration of ammonium chloride. However, in none of our patients did water retention lead to recognizable edema and ammonium chloride was administered in several cases. As mentioned on p. 541 the potassium balances probably do not reflect total pre-admission losses. If additional retention of potassium (during postoperative recovery) occurred in exchange for intracellular sodium, or with equivalent amounts of non-metabolizable anion such as phosphate, the effect on the NAB would be zero—and the pre-operative NAB representative of (total) pre-admission losses of net acid.

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I	21 671	4.801	5.556	11.314	9.891
II	−7 168	−0 728	0 479	−6 919	−1 087
III	4 796	3 712	2.372	−1.288	−0.198
IV	4.378	0 950	0.331	3 097	0.395
V	3 930	2 943	1 082	−0 095	0.211
VI	24.834	2.918	3 466	18 450	22 100
VII	10 481	3.841	2.383	4.257	5.525
VIII	0 406	0 798	0.320	−0 712	2.461
IX	12.554	4 749	2.638	5 167	6.322
X	1 170	−0 739	1 071	0 788	4 988
XI	1 419	2 117	1 610	−2.308	−0.235
Mean	7 179	2.260	1 937	2.932	4.579

$\Delta Na_{ECW} = 0.370 \times 140 - [Na^{+}]_{b} \times ECW_{b}$ for $[Na^{+}]_{b}$ see Table 1

$NAB = 1.906 + 0.912 (\Delta Na_{ECW} - B_{Na} - B_{K})$ $r = 0.94$ $p < 0.001$

cantly ($p<0.001$) correlated to the NAB (Table 6) The two right hand columns of the table suggest a one to one relationship between mmoles of extracellular sodium lost to non-soft tissues and mEqs of net base accumulated during the period of vomiting (regression coefficient 0 912) Deposition of sodium carbonate in the skeleton is an attractive possibility Actually recent studies in dogs have documented carbonate deposition in tibial cortical bone in experimental alkalosis (8)

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EFFECT OF DIFFERENT HUMAN GROWTH HORMONE PREPARATIONS ON NITROGEN RETENTION IN HYPOPITUITARY CHILDREN

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ABSTRACT Butenandt, O Bidlingmaier F and Knorr D. (Department of Paediatrics, University of Munich, Germany). Effect of different human growth hormone preparations on nitrogen retention in hypopituitary children Acta Paediatr Scand, 63-549 1974.—The biologic activity of three different preparations of human growth hormone (HGH) obtained with the methods of Raben, Reisfeld and Roos respectively were compared in nitrogen retention tests in 18 patients with hypopituitarism and in 17 children with familial shortness of stature. HGH Raben produced a mean nitrogen retention of 30.9% in 11 hypopituitary patients and no retention in the control group HGH Reisfeld was followed by retention of 19 7% in the 9 hypopituitary patients and an elevated excretion of 12% in the control group, and HGH Roos evoked a retention of 33.1% only in the 9 hypopituitary children. HGH Reisfeld had a lower response in consecutive tests with different HGH preparations in the same patients than the other two HGH preparations. The dilution curve of HGH Reisfeld obtained with a double antibody radio immunoassay did not parallel a dilution curve of a reference hormone from the National Institute of Health whereas the other two preparations had identical dilution curves. It is concluded that the method of extraction alters the biologic acti ity of the hormone as well as the physico-chemical properties.

KEY WORDS: Human growth hormone nitrogen retention, hypopituitarism

Human growth hormone for treatment of hypopituitarism can be extracted from human pituitary glands by different methods Laboratory investigations by Schleyer et al (1?) have shown differences between several preparations of human growth hormone (HGH) No comparative investigations on biologic activity of different HGH preparations in man have been performed. In the present study three hormonal preparations used for treatment of hypopituitarism are compared The first part deals with the radio-immuno-assayable content whereas in the second part the biologic activity in man during nitrogen retention tests is presented.

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METHODS AND MATERIALS

Growth hormone preparation

1 HGH "B" This preparation was prepared from acetone dried pituitary glands in the laboratory of Raben (9). 1 0 mg of the powder was dissolved in 0 03 ml of hydrochloric acid (0 05 n) and diluted with 0.97 ml of saline (0 9%). This solution was further diluted with borate buffer pH 8 4 0.13 Mol, containing 0.5% bovine serum albumin, to a final concentration of 10 ng powder/ml for radio-immunoassay

2. HGH "D" This preparation was obtained from Arzneimittelwerk Dresden (commercial name Sotropin H) and is obtained by the method of Reisfeld (10). The content of one ampulla (16 mg powder) was diluted with 2.0 ml of saline according to the instructions for clinical use. It was further diluted for the radio-immunoassay with the above mentioned buffer to a final concentration of 8 ng powder/ml.

3 HGH "M" This preparation was obtained with the method of Roos et al. (11) It is commercially available as "Crescormon" (Deutsche Kabi GmbH). One ampulla, containing 2.0 mg HGH was diluted with

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EFFECT OF DIFFERENT HUMAN GROWTH HORMONE PREPARATIONS ON NITROGEN RETENTION IN HYPOPITUITARY CHILDREN

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ABSTRACT. Butenandt, O., Bidlingmaier, F. and Knorr, D. (Department of Paediatrics, University of Munich, Germany). Effect of different human growth hormone preparations on nitrogen retention in hypopituitary children. Acta Paediatr Scand, 63: 549, 1974.—The biologic activity of three different preparations of human growth hormone (HGH) obtained with the methods of Raben, Reisfeld and Roos respectively were compared in nitrogen retention tests in 18 patients with hypopituitarism and in 17 children with familial shortness of stature. HGH Raben produced a mean nitrogen retention of 30.9% in 11 hypopituitary patients and no retention in the control group. HGH Reisfeld was followed by a retention of 19.7% in the 9 hypopituitary patients and an elevated excretion of 12% in the control group, and HGH Roos evoked a retention of 33.2% only in the 9 hypopituitary children. HGH Reisfeld had a lesser response in consecutive tests with different HGH preparations in the same patients than the other two HGH preparations. The dilution curve of HGH Reisfeld obtained with double antibody radio immunoassay did not parallel a dilution curve of a reference hormone from the National Institute of Health whereas the other two preparations had identical dilution curves. It is concluded that the method of extraction alters the biologic activity of the hormone as well as the physico-chemical properties.

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2 0 ml saline according to the instructions for clinical use A further dilution with the above mentioned buffer to a final concentration of 10 ng/ml was done for the radio-immunoassay

Radio-Immuno-Assay

The radio-immuno-assay was performed using the HGH kit from CEA-IRE-Sorin based upon a double anti body technique The HGH standard used in this test was prepared at the Institute of Clinical Physiology National Research Council Pisa Italy according to the method of Wilhelmi (17) In addition we used a refer ence standard from the National Institute of Health (HS 1394 2 IE/mg) again a Wilhelmi-preparation

Nitrogen retention test

The patients received a constant diet containing 15% of the caloric needs as protein 35% as fat and 50% as carbohydrates The total excretion of urea nitrogen in the urine was measured daily The first 5 days served as control period The mean amount of excreted urea nitrogen from day 2 to 4 was taken as control value From day 6 on for a total of 4 (up to 6) days the patients received 2 mg HGH daily intramuscularly The mean daily urea nitrogen excretion from days 7 8 and 9 was taken for calculation of the retention of nitrogen as follows

$$\text{Nitrogen retention (\%)} = \frac{(U_c - U_H)\,100}{U_c}$$

U_c=mean daily urea nitrogen excretion during first period (control period) U_H=mean daily urea nitrogen excretion during second period (on growth hormone)

Patients

Nitrogen retention tests were performed in 18 patients with hypopituitarism and in 17 patients with familial dwarfism evaluated to have normal production of growth hormone Hypopituitarism might be due to birth trauma (6 patients) trauma by accidents (3 patients) tumors in the hypothalamo-hypophyseal region i e cranio-pharyngeoma (4 patients) congenital brain defect (1 patient) 4 patients had idiopathic hypopituitarism

The diagnosis of growth hormone deficiency was based upon measurements of plasma growth hormone following a dose of 0 1 IU insulin per kg body weight No measurable increase of plasma growth hormone following a significant fall of blood glucose was found in hypopituitarism though a good response (increase of more than 7 ng/ml serum) was present in the other children

RESULTS

Table 1 summarizes the results of the deter minations of the hormonal content of the three preparations performed 5 times on each preparation separately According to our findings 1 mg HGH B powder was comparable to 0 7 mg of the HGH standard One ampulla HGH M contained 2 06 mg of HGH and one ampulla of HGH D 4 8 mg (mean values) However taking into consideration the different dilution curve of HGH D (see below) the last value is true only for the dilution as described above The table also shows the data given by the phar maceutical companies

In a further experiment different dilutions have been made from each preparation As shown in Fig 1 the dilution curves of HGH B and HGH M are in accordance with the dilution curve of the HGH-standard as well as to that of the NIH-reference hor mone The dilution curve of HGH D does not parallel the standard curve

Table 1 *Immunoassayable content of different HGH preparations*

HGH-preparation	HGH "B"	HGH "M"	HGH "D"
Investigated amount	10 mg powder	1 Ampulla	1 Ampulla
Stated content	–	*mg*=4 IU	10 USP-U
Measurable content of HGH (single determinations)	7 3 mg	2.1 mg	5 4 mg
	7 0 mg	2.0 mg	5 0 mg
	7 4 mg	2.0 mg	4.3 mg
	7 0 mg	2.1 mg	5 0 mg
	6.8 mg	2.1 mg	4 6 mg
Mean HGH-content	7 1 mg	2 06 mg	4 8 mg

Nitrogen retention test

Eleven patients (mean age 10 years range 5–15 years) receiving HGH B had a nitrogen retention between 16.5 and 49% (mean 30 9%) Eight of these patients showed a retention of more than 25% (Table 2) On the fourth day of administration of HGH (day 9 of the test) the mean nitrogen reten-tion was 39 2% however on this single day one patient had a retention of only 8% The range of all patients was 8 to 75% retention

The mean nitrogen retention of 9 patients (mean age 10 years range 4–14 years) re

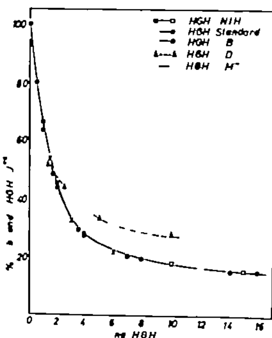


Fig 1 Dilution curve of different preparations of human growth hormone Whereas the preparations B" (Raben) and "M (Roos) have the same dilution curve as the HGH standard (preparation from Sorna) and the reference HGH (NIH.HS 1394 2 IU/mg) the preparation D (Reisfeld) has a different dilution curve

ceiving HGH M was 33 2% the range being 0 to 54.5% One patient (H S) did not show any change of the nitrogen excretion at all In 7 patients the nitrogen retention exceeded 25% On the fourth day of HGH administration the mean nitrogen retention was 34 7% the range being 13 0 to 62 0%

The least retention of urea nitrogen was found in the group of 9 patients (mean age 10 years range 4–15 years) treated with HGH D" The range of the test results was between an increase of urea nitrogen excretion of 9% and a retention of 45% the mean being 19 7% retention. Only 3 patients had a retention of more than 25% (Table 2). On the fourth day of hormonal treatment the mean retention of urea nitrogen was 24.3% the range between 0 and 44%

In 11 patients at least two different preparations of HGH were tested separately The

Table 2. *Nitrogen retention tests in hypopituitary patients*

HGH-preparation	HGH "B	HGH "M"	HGH "D"
Number of patients treated with each preparation	11	9	9
Nitrogen retention (%)			
Mean of 3 days ±S. D	30.9±10 6	33_±14 8	19 7±17.3
Range	16 5–49 0	0 –54.5	–9 0–45.0*
Day 4 (mean)	39.2	34 7	24.3
Range	8 0–75 0	13 0–6_0	0 –43.5

Negative retention = increased excretion.

diet was not changed at all during the two tests Four patients received HGH B and HGH D the first preparation produced a mean nitrogen retention of 30 7% whereas the other one had no effect in 2 patients and a good one in the two other patients However compared with the result obtained with the first preparation, the effect produced by HGH D" was smaller also in these two patients (Fig. 2 Table 3)

Six patients received HGH D and HGH M" during subsequent metabolic studies HGH D was followed by a nitrogen retention of 21 6% (range between 8% in-

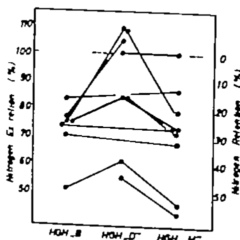


Fig 2 Nitrogen excretion during administration of 2 mg HGH daily in 11 hypopituitary patients. The results of different nitrogen retention tests in the same patient are connected by solid lines. There is less nitrogen retention during the administration of HGH "D" compared with HGH "B or "M

Table 3 *Comparison of nitrogen retention in different tests under different HGH preparations in the same patients*

Pat	(sex)	age y	Mean nitrogen retention (%) HGH B"	HGH M"	HGH D
D E.	(m)	6	25 0		−9 0*
W W	(m)	10	23 7		4 0
A K	(m)	6	25 0	27 0	16.0
D B	(m)	14	49 0	54.5	39 0
U X.	(m)	13	16 5	13 0	
A H	(f)	9	76.0	77 0	
G E	(m)	12	30 0	32.7	
H S	(m)	4		0	0
S I	(f)	4		58 0	45 0
P K	(f)	13		21 0	−8 0*
J R.	(m)	8		28 7	16 0

p (B / M ") n s. p (B or M / D ") 0 02
* Negative retention = elevated excretion

creased excretion and 45% retention) whereas HGH M evoked a nitrogen retention of 37 7% (range 21 to 58%) in the same patients There was no difference on day 4 of HGH administration between the two preparations whereas HGH D produced a retention of urea nitrogen of 30% (mean) the preparation M produced one of 33% One patient (H S) had no changed metabolism at all the mean nitrogen excretion being unchanged during both tests

The 5 patients receiving either HGH B or HGH M did show only minor differences in their response to either one of the two preparations (Fig 2)

The healthy children receiving growth hormone did not show significant changes of urea nitrogen excretion Ten children (mean age 11 0 years range 5–15 years) received HGH B The mean nitrogen excretion was 101% of the mean control excretion (that means no retention of urea nitrogen) The range was 8% retention (=92% excretion) to 112% excretion compared with the control period = 100% (Table 4)

Also 5 children (mean age 10 years) receiving HGH D had no change of the urea nitrogen excretion or excreted even more urea nitrogen during the days they received HGH compared with the first period The mean nitrogen excretion was 112% (range 103 to 127%)

So far only two metabolic tests have been performed with HGH M in boys with normal pituitary function They had a nitrogen retention of only 2 and 8%

DISCUSSION

Noteworthy differences of different growth hormone preparations were found in investigations by Schleyer et al (12) They stated that a preparation of Roos had 86% a preparation of Raben 49% and a preparation of Reisfeld only 10% content of HGH compared with a self-made standard However it is not stated whether this is related to the protein content of the preparations or to any other basis

We found a slight difference of the slope obtained by diluting the different preparations HGH D did not parallel the standard curve whereas the other preparations did Werder et al have shown that in such a case the deviation from the standard curve may be due to a different hormone with partial immuno-identity or to changes of the antigenic site The same authors also demonstrated that different growth hormone preparations had identical dilution curves as a standard preparation (16) On polyacrylamid gel electrophoresis a HGH standard from the National Institute of Health prepared with the method of Wilhelmi (17) appeared as one single band whereas preparations of Roos and Raben were separated

Table 4 *Nitrogen retention tests in children with normal growth hormone production*

HGH preparation	HGH B	HGH M	HGH D"
Number of children treated with each preparation	10	2	5
Range of nitrogen retention (%)	−12 0–8 0	0–8.0	−27 0– −3 0*
Mean nitrogen excretion	100 8%	95%	112%
Retention	None	5%	−12%*

* Negative retention = elevated excretion.

nto three bands A preparation of Reisfeld inally showed three thick and another broad band near the cathodic end (14) Using Sephadex gel filtration, fast moving HGH material is suggestive of aggregation of mole cules whereas slow moving polypeptides indicate proteolysis of HGH (13) Schleyer et al (12) too demonstrated a different behaviour of the Reisfeld preparation on gel filtration, indicating that this preparation has a slightly lower molecular weight than other preparations i e preparations Roos or Raben

The anabolic action of growth hormone is a well known phenomenon (1 4 7 15) and the use of the nitrogen retention test by Prader et al (8) for diagnostic purposes has been accepted by several investigators (2, 5 7). Total nitrogen excretion as well as urea nitrogen excretion has been taken as criterium for the protein metabolism in man and a diminished excretion of one of these parameters indicates synthesis of protein Thus it seems justified to use the nitrogen retention test for investigations of the somatotrophic effect of different preparations of HGH

The different results between growth hormone deficient patients and healthy children again demonstrate the diagnostic value of the test. However some of the patients did not respond well to HGH during this short time of administration This reduces the value of the procedure for diagnostic purposes on the other side Hubble (5) calls the nitrogen retention test a valuable help for diagnosing growth hormone deficiency But he suggests to use a higher dose of HGH than we have done in the present study A better result can possibly be obtained also in the patients who did not respond to 2 mg of the hormone

The three different preparations used in our hospital did not show the same biologic activity in children when given in the same quantity The best result was obtained with the preparation of Roos (HGH M) closely followed by that of Raben (" B") The third preparation (HGH D ') had no effect on the mean nitrogen excretion in 3 of 9 patients and an equivocal effect (16% retention) in 2 more patients Using the preparation of Raben only one out of the 11 patients with hypopituitarism showed a questionable effect of 16.5% retention whereas the other patients had a retention of more than 20% In a bigger series of 32 patients with lack of endogenous growth hormone production one did not respond to HGH B and 4 responded weakly (3) Under administration of HGH M" one out of 9 patients had a weak retention of 3% only Statistically the mean values of the three groups did not differ significantly

These differences in biologic activity may be due either to the method of extraction of the hormone or due to differences in the individual response The latter may be the explanation for the weak response of some patients to either one of the 3 preparations even during consecutive tests

Other patients however receiving two different preparations did show different responses thus demonstrating a different biologic activity of the preparations Statistically the different responses following administration of preparation M or B is not significant at all If present The lesser nitrogen retention following preparation D" compared with the two other preparations is significant ($p=0.02$) As shown by laboratory investigations the preparation of Reisfeld differed also from other preparations (12) However it may be possible to obtain a better result in increasing the amount of daily injected hormone as suggested by Hubble (5) Further evaluation of the therapeutical value of the preparations during longterm therapy of hypophyseal nanism is necessary for answering this question.

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THE PROGRESSIVE ANTITHROMBIN ACTIVITY AND ITS RELATIONS TO OTHER FACTORS OF THE COAGULATION SYSTEM IN NEWBORNS

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ABSTRACT Weissbach, G. Domula, M., Lenk, H. and Schneider P (Paediatric Clinic of the Medical Department, Karl-Marx-University Leipzig DDR) The progressive antithrombin activity and its relations to other factors of the coagulation system in newborns. Acta Paediatr Scand, 67: 555 1974.—The progressive antithrombin and its relations to other factors of the coagulation system have been studied in chronically asphyxiated newborns. The progressive antithrombin activity was determined in 44 healthy and in 40 chronically asphyxiated newborns according to the method described by Gerendas & Monkhouse The activity was clearly depressed in the asphyxiated group as compared with healthy full-term newborns. Only within the group of asphyxiated newborns did the statistical analysis reveal close correlations between progressive antithrombin and the factors fibrinogen, plasminogen, thromboplastin time value, factor II, and thrombocytes. Furthermore most of these were also closely correlated among themselves. The partly very close relations can be explained only by a simultaneous consumption of these constituents by disseminated intravascular coagulation processes and secondary hyper fibrinolysis. The decrease in progressive antithrombin is due to an irreversible binding of the antithrombin III to thrombin, liberated within the vessels.

KEY WORDS: Progressive antithrombin

The progressive antithrombin activity of the human plasma mainly comprises two inhibitors. the major part is antithrombin III the minor part the α_2-macroglobulin (1 7 24 33 34 35) Antithrombin III binds thrombin irreversibly The heparin co-factor formerly called antithrombin II and antithrombin III are identical (17 24 34 39) Additional to the progressive antithrombin the total antithrombin potential comprises further factors fibrinogen (antithrombin I) and under certain conditions fibrin(ogen) degradation products (antithrombin VI)

The progressive antithrombin is reduced in healthy newborns (6 9 17 18 30 37 42) Exact data on the behaviour of the progressive antithrombin in newborns with chronic asphyxia have so far not been available It is one aim of this study to provide informations on this subject.

Severe consumption coagulopathies so-called defibrination syndromes are no rare observation in newborns with chronic asphyxia (43) In chronically asphyxiated newborns close correlations exist between the components plasminogen fibrinogen factor V and thrombocytes (41) This can be explained only by their intravascular consumption. Thus chronic asphyxia in newborns is frequently or even regularly associated with disseminated intravascular clotting processes Other authors (3 10 15 16 19 22 36) had also come to these conclusions owing to coagulation analyses Antithrombin III is consumed by disseminated intravascular

These studies are part of the medical research programme "Perinatology" of the Ministry of Health of the DDR

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ABSTRACT Weissbach, G., Domula, M., Lenk, H. and Schneider P (Paediatric Clinic of the Medical Department, Karl-Marx-University Leipzig, DDR) The progressive antithrombin activity and its relations to other factors of the coagulation system in newborns. Acta Paediatr Scand, 63: 555, 1974.—The progressive antithrombin and its relations to other factors of the coagulation system have been studied in chronically asphyxiated newborns. The progressive antithrombin activity was determined in 44 healthy and in 40 chronically asphyxiated newborns according to the method described by Gerendas & Munkhouse. The activity was clearly depressed in the asphyxiated group as compared with healthy full-term newborns. Only within the group of asphyxiated newborns did the statistical analysis reveal close correlations between progressive antithrombin and the factors fibrinogen, plasminogen, thromboplastin time value factor II, and thrombocytes. Furthermore most of these were also closely correlated among themselves. The partly very close relations can be explained only by a simultaneous consumption of these constituents by disseminated intravascular coagulation processes and secondary hyper fibrinolysis. The decrease in progressive antithrombin is due to an irreversible binding of the antithrombin III to thrombin, liberated within the vessels.

KEY WORDS: Progressive antithrombin

The progressive antithrombin activity of the human plasma mainly comprises two inhibitors: the major part is antithrombin III the minor part the α_2-macroglobulin (1 7 24 33 34 35) Antithrombin III binds thrombin irreversibly The heparin co-factor formerly called antithrombin II and antithrombin III are identical (17 24 34 39) Additional to the progressive antithrombin the total antithrombin potential comprises further factors fibrinogen (antithrombin I) and under certain conditions fibrin(ogen) degradation products (antithrombin VI)

The progressive antithrombin is reduced in healthy newborns (6 9 17 18 30 37 42) Exact data on the behaviour of the progressive antithrombin in newborns with chronic asphyxia have so far not been available It is one aim of this study to provide informations on this subject.

Severe consumption coagulopathies so-called defibrination syndromes are no rare observation in newborns with chronic asphyxia (43) In chronically asphyxiated newborns close correlations exist between the components plasminogen fibrinogen factor V and thrombocytes (41) This can be explained only by their intravascular consumption Thus chronic asphyxia in newborns is frequently or even regularly associated with disseminated intravascular clotting processes Other authors (3 10 15 16 19 22, 36) had also come to these conclusions owing to coagulation analyses Antithrombin III is consumed by disseminated intravascular

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These studies are part of the medical research programme "Perinatology" of the Ministry of Health of the DDR.

coagulation (21 23) counteracting this very process Therefore the relations between the progressive antithrombin and other components of the coagulation system in chronically asphyxiated newborns are of utmost interest

MATERIAL AND METHODS

Coagulation analysis was carried out in 40 newborns with chronic asphyxia mostly on the first day in some instances on the second day of life and before the onset of any therapy with plasma derivatives and heparin. Those newborns were designated as chronically asphyxiated who had a severe respiratory distress syndrome with the symptoms of hypoxia and acidosis persisting for more than 30 minutes In 21 patients the birth weight was below 2 500 g in 19 it was more than 2 500 g A group of 44 healthy newborns up to the age of 2 days were used as controls

The following factors were determined in the patient s citrated plasma (1 vol 3.8% sodium citrate solution 9 vol blood)

1 Progressive antithrombin according to Gerendas & Monkhouse (24 27) We have used the Monkhouse method with a slight modification Our sample consisted of only 0 1 ml defibrinated plasma 0.3 ml Michaelis buffer pH 7 4 and 0 2 ml thrombin solution with 15 NIH U/ml in 0 006 M Tham buffer pH 8.5 The plasma samples were heated up to 56°C for 5 minutes Mostly we determined the coagulation time every minute in cases of low antithrombin activity at longer intervals In accordance with Monkhouse the coagulation times regularly showed a linear course when plotted in a semi-logarithmic coordinate system The slope angle of the line was determined with help of the tangent function The slope angles determined by plasma dilution again were situated on a linear curve when plotted in a logarithmic system against the degree of dilution Such calibration lines allow the transformation of slope angles determined for the patients plasma specimens into antithrombin activity values (% of the adult normal value) The calibration lines were always determined by the same standard plasma which consisted of a pool of 20 blood donor plasma samples kept in small volumes at −20°C.

2 Fibrinogen (mg %) as tyrosin equivalent according to Young & Kolmen (44)

3 Plasminogen (% of the adult normal value) as proactivator of bovine plasminogen according to Blix (8)

4 Thrombin time (sec.) adult normal value 20± 2 sec

5 Thromboplastin time value (% of the adult normal value)

6 The factors II V and X (% of the adult normal value) with the reagents of the Behringwerke

The thrombocyte count (per μl) was determined by the phase-contrast method in the venous blood of the patients

Table 1 *Mean values of progressive antithrombin and other components of the coagulation system*

	Healthy full-term newborns		Newborns with chronic asphyxia	
	n	$\bar{x}$	n	$\bar{x}$
Progressive antithrombin %	44	79.34	40	47.85
Fibrinogen mg/100 ml	44	254.55	37	202.49
Plasminogen %	44	51 64	40	30 95
Thrombin time sec	44	29.34	39	32 69
Thromboplastin time value %	44	42 05	38	36.50
Factor II %	43	35 81	37	29 03
Factor V %	41	64 78	36	42.36
Factor X %	43	[illegible]3 44	37	22 73
Thrombocyte count 1000/μl	35	237 74	39	115 69

The following statistical values were calculated. mean value ($\bar{x}$) standard deviation (s) standard error ($s_{\bar{x}}$) regression coefficient correlation coefficient according to Bravais (r) and partial correlation coefficients. The following random critical tests were carried out Identity test of random samples with the χ^2-test as homogeneity test according to Brandt Snedecor test of regression and correlation coefficients with null hypothesis and test of the difference between the correlation coefficients (40).

RESULTS

Table 1 shows the mean values n indicates the random size The progressive antithrombin activity of healthy full term infants amounts to a mean of 79 34% (s=14 99% $s_{\bar{x}}$=2 26%) and that of chronically asphyxiated to 47 85% (s=18 05% $s_{\bar{x}}$=2 85%) of the adult normal value The two distribution profiles (Fig 1) show a considerable difference According to the χ^2-test they are different with an error probability α of 0 1% If the group of asphyxiated newborns is subdivided according to birth weight the group below 2 000 g has a much lower mean value ($\bar{x}$=39 14%) than the group above 2 000 g ($\bar{x}$=57 47%) The χ^2 test indicates a difference of these two distributions with an error probability of 2 5% However the distribution of the antithrombin values of asphyxiated newborns with a birth weight above 2 000 g differs significantly (α=0 1%) from that of healthy full term newborns

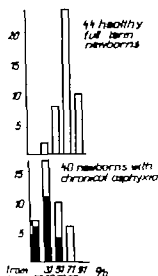


Fig 1 Distribution of the progressive antithrombin activities in 44 healthy (*top*) and 40 chronically asphyxiated newborns (*bottom*). The values of healthy full-term infants are clearly below adult normal, the values of asphyxiated below those of healthy full-term infants. ■ Distribution of the values of patients with a birth weight less than 000 g, which are concentrated in the lowest group.

The mean values of fibrinogen plasminogen, thromboplastin time value factor V and thrombocytes calculated for the group of chronically asphyxiated are considerably lower than those of the healthy full-term group

Table 2 shows the results of the correlation analysis In the random critical test the correlation coefficients must exceed the random maximal values which are given by the number of degrees of freedom and by the error probability α Thus α indicates the degree of dependence between the factors The correlation is to be regarded as very close with $\alpha \leq 0.1\%$ as close with $\alpha \leq 1.0\%$ and as weak with $\alpha \leq 5.0\%$ The right hand column of Table 2 shows the results of the random critical test of the difference between the correlation coefficients of healthy (r) and chronically asphyxiated newborns (r_b)

In healthy newborns the progressive antithrombin is only weakly correlated with the factors plasminogen and factor V and closely with the thrombocyte count However in asphyxiated infants progressive antithrombin is correlated with all factors examined. A very close positive correlation exists to the factors fibrinogen, plasminogen and factor II a close positive to the thromboplastin time value and to the birth weight, a close negative to the thrombin time. The relation to factor V factor X and thrombocytes is weak The difference between the correlation coefficients of healthy and asphyxiated proves significant for the relations of the progressive antithrombin to the factors fibrinogen plasminogen, thromboplastin time value and factor II

Table 2 *Results of the analysis of correlations between progressive antithrombin and other parameters*

Relations of progressive antithrombin to the factors	(a) Healthy full-term newborns			(b) Newborns with chronic asphyxia			Difference ↔
			α, %	n		α, %	(α%)
Fibrinogen	44	−0.021	>5	37	0.521	0.1	2
Plasminogen	42	0.355	5	40	0 773	0.1	1
				40	0.731*	0 1	
Thrombin time	44	−0 .33	>5	38	−0 409	1	50
Thromboplastin time value	44	−0.207	>5	38	0 410	1	1
Factor II	43	−0.059	>5	37	0.537	0 1	1
Factor V	41	0 334	5	36	0.408	5	>50
Factor X	43	0.024	>5	37	0.371	5	25
Thrombocytes	35	0.422	1	39	0 334	5	>50
Birth weight				40	0 460	0.27	

* Partial correlation coefficient: elimination of the variable birth weight

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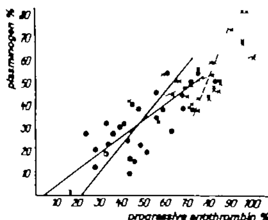


Fig. 3. Relation between progressive antithrombin and plasminogen. Symbols are the same as in Fig. 2. As the regression lines show factors are very closely related in chronically asphyxiated (–) but only loosely related in healthy full-term newborns ( ). The scatter diagrams are connected without interruption. Asphyxiated newborns with a birth weight less than 2 000 g show the lowest values of progressive antithrombin and plasminogen.

we used is based on low doses of thrombin. Subject of the determinations were the initial rates of thrombin inactivation. This procedure is more appropriate for the *in vivo* situation than methods diagnosing the total thrombin binding capacity of the plasma by means of high thrombin doses (2). However fibrinogen degradation products also interfere with the results of this procedure. Therefore we first heated up the plasma samples to 56°C for 5 min. In this way fibrinogen degradation products with antithrombin and anti-polymerase property are eliminated together with fibrinogen (5). The instant antithrombin property of heated plasma samples of healthy and chronically asphyxiated newborns shows unimportant differences (42). Largely false results due to the peculiarities of newborns need not be expected with this procedure.

In chronically asphyxiated newborns the progressive antithrombin activity was considerably below the values of healthy newborns. There were numerous premature infants with birth weights below 2 000 g in our collection. The progressive antithrombin correlated with the birth weight. The lower progressive antithrombin activity in the plasma of pre term infants is not necessarily due to a lower antithrombin III level. Abildgaard et al. (1) had verified that in adults antithrombin III is the most important component of the progressive antithrombin. In newborns possibly it is slightly more influenced by the α_2-macroglobulin. For this is considerably increased in healthy full-term infants as compared with the adult level (13, 14). Ekelund et al. (11, 12) found an increase of the α_2-macroglobulin in the blood of fetuses and premature infants with higher gestational age. The increase in α_2-macroglobulin might explain the increase of progressive antithrombin with higher birth weight. The explanation of this question however is only possible by the isolated determination of the components of the progressive antithrombin. The immaturity of some newborns cannot be the only nor even the most important cause of the decrease of progressive antithrombin in the group of chronically asphyxiated infants. For this is also significantly reduced in the sub-group of patients with higher birth weight when compared with the neonatal normal value.

In healthy full-term newborns the progressive antithrombin is closely correlated only with the thrombocytes and weakly with the factors plasminogen and factor V. The interpretation of the correlations is so far not possible. There were more and closer relations of the progressive antithrombin to other factors of the coagulation system in the newborns with asphyxia. The fibrinogen level is independent of the birth weight (3, 11, 15, 20, 25, 38). However a very close correlation exists between progressive antithrombin and fibrinogen. The extraordinarily close correlation to plasminogen is doubtlessly favoured by the dependence of both factors on the degree of maturity of the infant. Other authors (4, 11, 28, 31, 32) also found lower plasminogen values in prematures or proved their dependence on the gestational age respectively. The

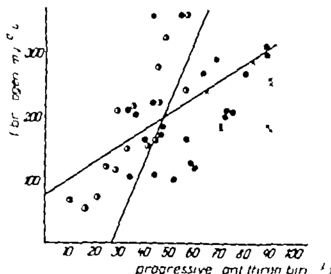


Fig 2 Relations between progressive antithrombin and fibrinogen ◐ chronically asphyxiated newborns (<2 000 g) ● chronically asphyxiated newborns (>2 000 g) × healthy full-term newborns The regression lines reveal the very close correlation between these two factors in chronically asphyxiated newborns The values of the patients with a birth weight less than 2 000 g are grouped in the left part of the scatter diagram that means in the lower progressive antithrombin activity

The very close connection between progressive antithrombin and fibrinogen in asphyxiated newborns is also documented in Fig 2 The values of the pre term infants below 2 000 g are grouped within the left part of the scatter diagram which means in the region of lower antithrombin activity as could be expected from the average values distribution profiles and correlation with birth weight However between fibrinogen and birth weight no mathematical relation exists ($r = -0.021$ $n = 38$) Classification according to birth weight does not provide differences of the mean values of fibrinogen In the subgroups progressive antithrombin is still rather closely correlated to fibrinogen but no longer to the birth weight (Table 3)

The extraordinarily close relation between progressive antithrombin and plasminogen in asphyxiated patients was also analysed in detail (Fig 3) The sub-groups of asphyxiated newborns below 2 000 g birth weight do not only comprise the lowest values of antithrombin activity but also the lowest plasminogen values In the total of asphyxiated patients plasminogens is weakly correlated to the birth weight ($r = 0.370$ $n = 40$ $\alpha < 5\%$) But the very close correlation between progressive antithrombin and plasminogen is constant even if the influence of the birth weight as a criterion of immaturity is eliminated Its partial correlation coefficient is still as high as 0 731 The correlations in the sub-groups formed by division of the total collection according to the birth weight are also very close (Table 3)

DISCUSSION

The progressive antithrombin activity was determined in the plasma of 44 healthy newborns and in 40 chronically asphyxiated newborns. We have used the method described by Gerendas & Monkhouse (24 27) In healthy full-term infants it amounted to an average of 79% of adult normal Thus the statement of the literature (6 9 17 18 30 37) that it is reduced in newborns is confirmed The discordant results communicated in the literature may be due to different methods Moreover some of these methods are not adequate concerning the general kinetics of reactions and the conditions of the neonate The method

Table 3 *Results of the correlation analysis in the subgroups*

Relations of progressive antithrombin to the factors	Chronically asphyxiated newborns					
	Birth weight < 000 g			Birth weight > 000 g		
	n	r	α%	n	r	α%
Fibrinogen	20	0 750	0 1	17	0 678	0 27
Plasminogen	21	0 765	0 1	19	0.699	0 1
Birth weight	21	0.201	>5	19	0 035	>5

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partial correlation coefficient and the close correlations in the sub-groups prove that the close connection between progressive antithrombin and plasminogen is not caused by immaturity The connection with the thrombocytes cannot be favoured by the factor of immaturity (14)

In asphyxiated patients the factors correlating with progressive antithrombin are also in a rather close relation among themselves (41) All these factors are reduced These correlations can be explained only by a common intravascular consumption of the factors comprising also constituents of the progressive antithrombin As the thrombocytes are involved in the pathomechanism one must assume intravascular consumption within the framework of a consumption coagulopathy with compensatory secondary hyperfibrinolysis Only disseminated processes of intravascular coagulation can create the close correlations between the factors in asphyxiated infants The intravascular liberation of thrombin is an essential step in the pathogenesis of such processes (23 29) Antithrombin III is bound by thrombin irreversibly This entails a reduction of the activity of progressive antithrombin In newborns with chronic asphyxia the thrombin time often is markedly prolonged (6 15 27 64) As one may see from the negative correlation between progressive antithrombin and thrombin time the prolongation of thrombin time in asphyxiated newborns can absolutely not be caused by an increase of antithrombin III The prolongation may be due to the effect of fibrin(ogen) degradation products with the progredient decrease in fibrinogen

In our opinion the cause of the frequent intravascular coagulation processes in chronically asphyxiated newborns are disturbances of microcirculation which are probably an almost regular event due to the influence of hypoxia and acidosis

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Fig 1 Smooth depressed "fracture" of the left parietal bone demonstrated on radiography



Fig 2 Elevation of depressed "fracture" effected.

DISCUSSION

Depressed fractures of the skull may interfere with the function and growth of the brain as well as establish an epileptogenic focus (1): early elevation is therefore necessary In the past surgery was thought to be the only recourse for such a condition (2) The advantages of an effective non surgical method of elevation are self-evident Two non-surgical methods of elevation have recently been described (3 4) In both reports the depressed fractures were obviously from recent trauma. Reduction was therefore more easily effected The two infants in the present report, had obviously sustained these depressions for some time as evidenced by their presence after easy deliveries and the absence of trauma in the overlying scalp. This impression was further confirmed by the ineffectiveness of the previously described methods in elevating the depression in Case 1 and the "flattening" that occurred after the first application in Case 2 It would seem therefore that the depressions had occurred *in utero*

The vacuum extractor would seem to be a very efficient instrument in effecting elevation of depressed fractures of the skull especially those that have been present for some time The amount of negative pressure created as well as the duration of application of this pressure can be varied to suit the situation Where necessary a second application can be performed with no ill effects The resultant oedema is only temporary None of the infants treated by this method in the Kandang Kerbau Hospital manifested any ill effects from this procedure The usual complications that attend a "vacuum extraction" delivery of an infant do not apply as no traction is applied in this situation Indeed this technique is ideally suited for reducing depressed fractures of the skull in the newborn especially those of already some duration.

Acta Pædiatr Scand 63 562–564 1974

ELEVATION OF CONGENITAL DEPRESSED FRACTURES OF THE SKULL BY THE VACUUM EXTRACTOR

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ABSTRACT Tan K. L. (Department of Paediatrics, University of Singapore Singapore) Elevation of congenital depressed fractures of the skull by the vacuum extractor Acta Paediatr Scand 63 562, 1974.—The vacuum extractor has been found to be effective in elevating depressed fractures of the skull, especially those of some duration. Two cases are reported to illustrate this procedure No ill effects have been encountered

KEY WORDS. Vacuum extractor depressed skull fractures

Congenital depressed fractures of the skull were noticed occasionally in newborn infants in the Kandang Kerbau Hospital Singapore These depressions had occurred in the absence of obvious trauma delivery had been uneventful and easy in the majority and no signs of trauma in the overlying scalp were observed In the past surgical elevation was necessary (2) It would however be preferable if elevation could be achieved without recourse to surgery For the past three years a non surgical method of elevation of such depressed fractures has been employed successfully in this Hospital It is the purpose of this paper to present two case reports that best illustrate this method of management

CASE REPORTS

Case 1

A male infant was delivered by Caesarean section after an uneventful full-term pregnancy The infant (birth weight 2800 g) cried lustily immediately after delivery and appeared normal except for a circular depression in the left parietal region 4 cm in diameter and 1 cm deep there was no trauma in the overlying scalp The head circumference was 34 9 cm. Radiology confirmed the presence of a depressed "fracture" (Fig. 1). It was thought that the "fracture" had occurred *in utero*

Two methods of non-surgical elevation have previously been described (3 4) these methods were attempted with no success A vacuum extractor was then used a medium sized cup (5 cm diameter) was placed over the depression and a negative pressure of 0.5 kg per cm² was maintained for a duration of 4 minutes. No traction was applied The infant remained well during the procedure With release of the pressure and removal of the cup a circular patch of oedema was observed Radiography immediately after the procedure demonstrated complete elevation of the depressed "fracture" (Fig. 2). The oedema disappeared within 5 hours revealing a normal head contour The infant's subsequent progress has been normal

Case 2

A female infant was delivered by the breech after 36 weeks gestation Delivery was uneventful the infant (birth weight ?013 g) appeared healthy at birth The head circumference was 32 cm A depressed "fracture" 4 cm by 3 cm with a depth of 0.75 cm was present over the left frontal bone there were no signs of obvious trauma in the overlying scalp

A vacuum extractor was used to elevate the dent a vacuum cup (diameter 4 cm) was placed over the depression and a negative pressure of 0.4 kg per cm² applied for 4 minutes The infant remained well during the procedure. The oedema subsided within 5 hours. Complete elevation was achieved at the first application. However a flattening of the area appeared on the second day after the procedure necessitating a second application permanent elevation was achieved after this The infant has since progressed normally

Acta Pædiatr Scand 63 565–570 1974

THE TRANSCAPILLARY ESCAPE RATE OF T 1824 IN NEWBORN INFANTS OF DIABETIC MOTHERS AND NEWBORN INFANTS WITH RESPIRATORY DISTRESS OR BIRTH ASPHYXIA

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ABSTRACT Ingomar C. Joh. and Klebe, J. G. (Diabetes Center of the Royal Maternity Hospital and the University Department for Newborn Infants, Rigshospitalet, Copenhagen, Denmark). The transcapillary escape rate of T 1824 in newborn infants of diabetic mothers and newborn infants with respiratory distress or birth asphyxia. Acta Paediatr Scand, 63: 565, 1974.—The influence of certain clinical conditions (idiopathic respiratory distress, birth asphyxia and diabetic embryopathy) on the transcapillary escape rate of human albumin, was investigated in 52 newborn infants. The dyestuff T 1824 (Evan's blue) was used for the labelling of plasma albumin in vivo, and its plasma concentration was determined spectrophotometrically using a micro-method. From serial measurements carried out during the first hour following the injection of T 1824 the escape rate (%/hour) was calculated. Among healthy newborn infants the escape rate was found to increase proportional to the magnitude of the placental transfusion. The same applied to infants with respiratory distress and infants of diabetic mothers, the escape rate of whom did not differ from that of healthy infants. By contrast, the escape rate of albumin was, among some cases of birth asphyxia, found to be increased out of proportion to the placental transfusion which the infants had received. It is discussed whether the increased escape rate found in these cases is caused by an increased capillary permeability or an increased capillary surface area.

KEY WORDS: T 1824, capillary permeability newborn infants, respiratory distress, asphyxia neonatorum, infants of diabetic mothers, placental transfusion

It is well-known that the dyestuff T 1824 (Evan's Blue) after injection into the blood stream is bound to plasma albumin. Its subsequent rate of disappearance is influenced by the capillary permeability the overall capillary surface area the rate of lymph drainage from the pericapillary space and the metabolic decomposition of albumin it may however under certain circumstances be an expression of the rate of *transcapillary* escape of albumin. This is the case when the disappearance rate of T 1824 is calculated from the fall in the concentration of the dyestuff during the first hour after its injection into the blood stream (3)

In a previous study not only was the transcapillary escape rate of T 1824 found to be higher in infants with late clamping of the umbilical cord compared with those with early clamping but also to increase proportionately to the volume of the placental transfusion (2) Since then we have examined newborn infants who were suffering from idiopathic respiratory distress syndrome (IRDS) infants who showed signs of birth asphyxia and infants who apart from being born of diabetic mothers were considered normal (IDM) Provided that the overall capillary surface area is unaffected by the presence of IRDS and birth asphyxia

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Table 3 *The transcapillary escape rate of T 1824 (%/hour) in various clinical groups Mean values±one standard deviation The influence of early (EC) and late (LC) clamping of the umbilical cord*

Numbers in parentheses indicate numbers of infants examined.

Group	EC	LC
1	19.6±4 7 (5)	25 0±4.3 (7)
2	17.5±4.6 (14)	–
3	16.8±3 4 (7)	26.7±5 9 (4)
4	25.6±7 7 (11)	–

consideration the type of clamping used however the average value of the rate of transcapillary disappearance of T 1824 was not found to differ in the four groups On the other hand if all non-asphyctic infants and all infants in whom IRDS was not manifest i.e group 1 and 2 were taken together and compared with those in whom birth asphyxia was in evidence the transcapillary rate of disappearance of T 1824 would be seen to be significantly higher in the latter ($p<0.02$ non-parametric test of Wilcoxon) due regard being taken to the type of clamping.

Measurement of the residual blood in the placenta gives an impression of the volume of the placental transfusion the smaller the volume the larger the placental transfusion. It appears from Fig. 1 that the rate of disappearance of T 1824 increases parallel with the volume of the placental transfusion. In this calculation the two groups viz. normal infants and infants born of diabetic mothers were dealt with collectively since the statistical analysis failed to show any difference in the slopes and levels of the two regression lines *TER 1824* (%/hour)=25 1−0 43×R (R=residual blood number of observations=26 $p<0.01$)

Fig. 2 shows that the rate of the transcapillary disappearance of T 1824 in newborn infants suffering from IRDS is proportionate to the placental transfusion they received In 5 out of 8 infants with birth asphyxia, however the actual rate of disappearance was found to be higher than expected on the basis of the placental transfusion alone

DISCUSSION

In earlier communications it has been demonstrated that the placental transfusion within the first hours after delivery is responsible for a loss of plasma into the interstitial space (2, 4 9) Such loss of plasma manifest in an increased haematocrit value can be explained by the assumption that the size of the intravascular space is too small at the

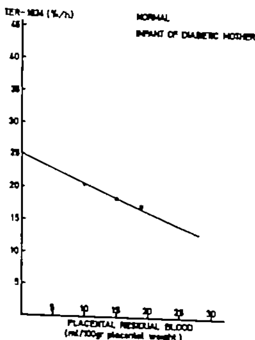


Fig 1 Scattergram and regression line Relationship between placental transfusion (see text) and transcapillary escape rate of T 1824 in newborn infants without birth asphyxia or respiratory distress.

Table 1 *Complications of pregnancy and delivery*

Group	No of cases	Pregnancy: Diabetic	Pregnancy: Placental insufficiency	Pregnancy: Other causes	Delivery: Foetal tachycardia or bradycardia	Delivery: Vaginal bleeding	Delivery: Caesarean section
1	12	–	1	–	1	–	–
2	16	16	1	–	1	–	12
3	11	2	3	3	1	7	4
4	13	5	3	1	2	2	5

and by the diabetic state of the mother an increased rate of the transcapillary escape of T 1824 would in these infants mean an increased capillary permeability It has been the aim of this investigation to throw light upon this problem

MATERIAL AND METHODS

The method used for the determination of the transcapillary escape of T 1824 was the same as that previously described (2 6)

The series consisted of 52 newborn infants who according to clinical findings were classified into four groups

Findings in the 12 normal infants comprising group 1 have been discussed in an earlier communication (2) these infants did not present any anomalies during the neonatal period. Group 2 is comprised of 16 infants born of diabetic mothers and who by and large did not present any abnormalities during the neonatal period. Group 3 (IRDS) is comprised of 11 infants who in the course of the neonatal period presented two or three of the following symptoms grunting retractions or tachypnoea. X-ray of the lungs revealed airbronchograms a reticular pattern of the lungs or diffuse blurring of the latter in 9 cases while the carbon dioxide tension of the capillary or arterial blood was found to be increased in 7 All infants survived in 5 cases after treatment with continuous positive airway pressure and/or intermittent positive pressure respiration Group 4 is comprised of 13 infants who showed signs of birth asphyxia, while the above-mentioned symptoms of IRDS were absent. According to Apgar score they were all asphyxiated Assisted ventilation for some minutes after birth was required in 7 cases Episodes of apnoea combined with cyanosis occurred in 5 in the course of the neonatal period other signs of cerebral anoxia were observed in two cases. All infants survived. It appears from Table 1 that complications which occurred during pregnancy or delivery were particularly numerous in the two last-mentioned groups and it appears from Table 2 that cases of premature delivery and low birth weight were more numerous in these groups than in the groups consisting of normal infants and infants born of diabetic mothers

In our report the term late clamping of the umbilical cord denotes clamping several minutes after birth early clamping denotes clamping as soon as possible after birth preferably within the initial 15 seconds after birth

In 40 cases the residual blood in the placenta was measured according to Redmond s method (8). The volume of the residual blood is expressed in ml per 100 gram of placenta

RESULTS

It appears from Table 3 that the rate of disappearance of T 1824 as previously reported (2) is higher in normal infants whose cords are clamped late than in those in whom clamping is performed early The same applies to infants with idiopathic respiratory distress syndrome Taking into

Table 2 *Clinical data Mean values*

Group	No of cases	Birth weight (grams)	Gestation (weeks)	Apgar score 1-min	Apgar score 5-min	Age (hours)	Clamping of the umbilical cord: Early	Late	Uncertain
1	12	3 070	39	9	10	4	5	7	–
2	16	3 552	37	9	10	14	14	–	2
3	11	2 380	34	7	9	19	7	4	–
4	13	2 665	36	4	8	14	11	–	2

cannot be decided on the basis of these findings If the latter is true however such increased dilatation must also be in evidence in organs other than the skin which is known to present clinical signs of poor circulation in cases of asphyxia

ACKNOWLEDGEMENTS

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The Editors have asked ass professor I Kjellmer University of Göteborg to comment on this article

In this issue of the journal a study from Copenhagen on the transcapillary escape rate of T 1824 in newborn infants of diabetic mothers and newborn infants with respiratory distress or birth asphyxia is presented. This study and the conclusions drawn by the authors raise several points of general interest The study of the diminution of the intravascular concentration of a macromolecule tagged with dye offers several difficulties in the interpretation of the results

The authors stress in their introduction that the disappearance rate of albumin from the intravascular compartment is influenced by the capillary permeability the overall capillary surface area, the rate of lymph drainage and the catabolism of albumin However the interpretation of the results rests on the assumption that "the overall capillary surface area is unaffected by the presence of IRDS and birth asphyxia and by the diabetic state of the mother" The suggestion of an unchanged capillary surface is even under ordinary physiological circumstances a bold assumption and becomes in the unsteady states studied by the authors merely an idle wish.

The crucial point in the discussion is the interpretation of the term overall capillary permeability The passage of macromolecules from the intravascular to the interstitial compartment depends on the characteristics of the capillary wall and the capillary surface area available for exchange Unfortunately these two features vary from tissue to tissue The permeability for macromolecules varies from the very restricted passage found in the blood capillaries of the central nervous system over the situation found in e g. skeletal muscle and skin to the almost nonrestricted passage for macromolecules typical for the hepatic circulation

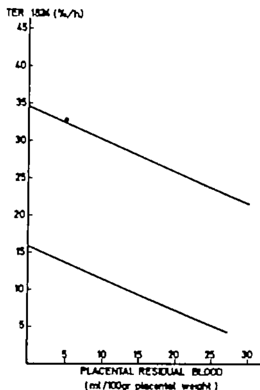


Fig 2 Relationship between placental transfusion (see text) and transcapillary escape rate of T 1824 95% confidence interval of normal infants. O newborns with idiopathic respiratory distress ● newborns with birth asphyxia.

time of delivery to hold the volume of the placental transfusion We also demonstrated a relatively greater loss of plasma in infants of diabetic mothers whose high birth weight is mainly attributable to the presence of fat (4 5) This phenomenon was explained by the fact that fat has relatively few vessels so that the reserve vascular capacity is inevitably relatively small Furthermore it has been demonstrated that the placental transfusion in normal infants is responsible for a dilatation of the capillaries of the skin probably due to the increased volume of blood as a consequence of which the overall surface area of the capillaries will be increased In cases of early clamping the infants concerned will not receive a placental transfusion at birth and the volume of plasma will usually remain constant during the first few hours after birth also the above-mentioned anatomical changes of the skin capillaries are far less pronounced (7)

On the basis of these findings we therefore conclude that the placental transfusion in normal infants may increase the transcapillary escape rate of T 1824 by way of an increase in the overall surface area of the capillaries

This feature was also seen in infants of diabetic mothers The values of the trans capillary escape rate of T 1824 in these cases were found to parallel those observed in infants born of normal mothers taking into consideration the volume of the placental transfusion These findings are in conformity with the clinical finding that the colour of infants born of diabetic mothers may often be rather high Assuming that the reserve vascular capacity in these infants is rather low the dilatation of the existing capillaries will be more pronounced than in infants born of non-diabetic mothers However the overall surface area of the capillaries need not be increased and thus the rate of transcapillary disappearance of T 1824 will be identical

In infants suffering from the idiopathic respiratory distress syndrome the rate of transcapillary disappearance of T 1824 was in all cases found to correspond to the amount of the placental transfusion they received Accordingly we are of the opinion that an increased capillary permeability need not be in evidence in these infants Our findings corroborate those obtained in another investigation where the rate of trans capillary disappearance of T 1824 was found to be identical in newborn infants whether or not they were suffering from the idiopathic respiratory distress syndrome (1)

On the other hand in most of the infants with birth asphyxia the rate of disappear ance was found to be higher than could be explained on the basis of the placental trans fusion alone Whether the capillary perme ability may have been increased in these cases or whether the overall surface area of the capillaries may have been increased by factors other than the placental transfusion

cannot be decided on the basis of these findings If the latter is true however such increased dilatation must also be in evidence in organs other than the skin which is known to present clinical signs of poor circulation in cases of asphyxia.

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The authors stress in their introduction that the disappearance rate of albumin from the intravascular compartment is influenced by the capillary permeability the overall capillary surface area the rate of lymph drainage and the catabolism of albumin. However the interpretation of the results rests on the assumption that "the overall capillary surface area is unaffected by the presence of IRDS and birth asphyxia and by the diabetic state of the mother" The suggestion of an unchanged capillary surface is even under ordinary physiological circumstances a bold assumption and becomes in the unsteady states studied by the authors merely an idle wish.

The crucial point in the discussion is the interpretation of the term overall capillary permeability" The passage of macromolecules from the intravascular to the interstitial compartment depends on the characteristics of the capillary wall and the capillary surface area available for exchange Unfortunately these two features vary from tissue to tissue The permeability for macromolecules varies from the very restricted passage found in the blood capillaries of the central nervous system over the situation found in e g. skeletal muscle and skin to the almost nonrestricted passage for macromolecules typical for the hepatic circulation

Capillary permeability to macromolecules thus demonstrates a wide range of variation between different tissues On the other hand available experimental evidence demonstrate that for any given tissue the permeability characteristics of the capillary wall are remarkably little influenced by changes of function—with few exceptions The number of capillaries open to blood flow (determining the functioning capillary surface area) stays constant in some organs (e g kidney and brain) but varies widely in other tissues according to the functional state (e g. skeletal muscle and skin) Therefore the terms capillary surface area and capillary permeability are meaningful first when related to one specified organ or tissue The terms overall permeability and capillary surface area lack this quality

The finding of an increased transcapillary escape rate for albumin can thus be equally well explained by an increased blood flow going to the liver circulation which will without any change of the characteristics of any capillary wall increase the leak of proteins from the circulation

Ingemar Kjellmer

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DOWN S SYNDROME IN SWEDEN

An Epidemiological Study of a Three-year Material

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ABSTRACT Lindsjö, A. (Department of Drugs, National Board of Health and Welfare Stockholm, Sweden). Down's syndrome in Sweden – an epidemiological study of a three-year material. Acta Paediatr Scand, 63: 571 1974.—A continuous, exact monitoring of the incidence of congenital chromosomal disorders is of great interest for the study of the possible significance of exogenous agents in the origin of various chromosome aberrations. An investigation of the incidence of Down s syndrome in Sweden has been carried out covering the years 1968–70 The total incidence (1:755) was found to be lower than in earlier Swedish investigations. This is probably due to reduction in the mean maternal age at childbirth. The earlier characteristic bimodal distribution of maternal age at the birth of children with Down's syndrome has now been reversed. The age-specific incidences showed significantly higher values for the maternal age groups 20–29 and more than 40 years in comparison with two earlier extensive surveys from the United Kingdom and Australia. A higher confirmation efficiency due to reports from cytogenetic laboratories may also possibly have contributed to the higher Swedish figures. From 1971 onward, Down's syndrome will be reported to the Swedish register of congenital malformations, so that changes in the incidence can be continuously observed. The investigation demonstrates the difficulty in comparing incidence figures from different time periods of a disease, the origin of which is dependent on maternal age.

KEY WORDS: Down's syndrome incidence, maternal age effect

The etiology of chromosomal aberrations is generally unknown Maternal age at conception is the only factor known to be of etiological significance for trisomy 21 Down s syndrome A relationship with X irradiation of the gonads (18) background irradiation (16) and viral infections especially hepatitis (10) has also been suggested, but definite evidence in favour of any of these hypotheses is so far lacking. Patients with Down s syndrome and their mothers have been reported to have higher titres of circulating thyroid antibodies than subjects in a corresponding normal population (4 14) The reason for this finding is not known. Disorders in ovarial function and especially overmaturity of ova may give rise to chromosomal disorders in animals and a similar situation may exist also with regard to Down s syndrome (6) It may also be mentioned that Carr (1) reported an increased frequency of triploidy among abortions in mothers who had ceased to take oral contraceptives less than 6 months before conception The frequency of different trisomies and X-monosomy was not increased however A direct relation between oral contraceptives and Down s syndrome has not yet been reported in the literature (7 8 9 19) An increased incidence of chromosome breakage and satellite associations was reported in blood cell cultures from 23 patients using oral contraceptives (11)

Numerous other environmental factors may

Table 1. *Number of Mb Down children born in Sweden 1968–70*

	Boys	Girls	Total	Total number of live born children	Incidence
1968	94	71	165	113 087	1:685
1969	70	61	131	107 662	1:8[illegible]1
1970	78	64	14[illegible]	110 150	1:776
Total	[illegible]4	196	438	330 859	1:755
	55.3%	44.7%			

affect the incidence both of Down's syndrome and of other conditions caused by chromosome aberrations and gene mutations. The aim of the present work was to obtain an up-to-date and as exact as possible survey of the incidence of Down's syndrome in Sweden for comparison with previous materials. At the request of the Swedish National Board of Health and Welfare an inventory of the incidence of Down's syndrome in 1968–70 has been made. Since January 1st 1971 it has been obligatory that cases of Down's syndrome shall be included among those conditions to be reported to the Swedish register of congenital malformations of the National Board of Health and Welfare. Thus, since that date a more careful supervision has been maintained by means of this register.

MATERIAL AND METHODS

The investigation was carried out in the spring of 1971 when records of children with the diagnosis of Morbus Down born in the years 1968, 1969 and 1970 were requested from all pediatric and gynecological departments, all boards for provisions and services to the mentally retarded and all cytogenetic laboratories in the country. The cytogenetic laboratories contributed 25% of the cases which had not been recorded via the other channels. This contribution was evenly spread throughout the maternal age-classes with one exception—the age group above 45 years in which only 16% new cases were added. Only cases with a positive chromosome analysis or with an unambiguous clinical picture were included. Of 473 collected cases 35 were excluded owing to a false diagnosis or incomplete documentation of the diagnosis. Data on the total number of children born in Sweden during the same period were obtained from the Central Bureau of Statistics.

RESULTS

The number of liveborn children with Down's syndrome reported in the years 1968, 1969 and 1970 was 438. 83.3% (365) were cytogenetically verified. Of these 365 patients 336 (92.1%) had trisomy 21, 10 (2.7%) an unbalanced D/G and 8 (2.2%) an unbalanced G/G translocation, while 11 (3%) demonstrated a 46/47, +21 mosaicism. The distributions of the cases according to year, sex and incidence for the whole material are shown in Table 1. Table 2 shows the distribution of all the liveborn children with Down's syndrome according to maternal age. At the date of birth of the child 46% of the mothers were below 30 years of age and 21% above 40 years, the median age being 31 years. During the same period the median age of childbearing Swedish women was 25 years and 78% of the mothers were below

Table 2. *Risk of birth of Mb Down children in different age groups 1968–70*

Mother's age	Mongoloid children: Number *n*	%	Live-born: *n*	% of all births	Incidence	%
Below 19 years	18	4.1	303[illegible]5	9.[illegible]	1:1685	0.059
20–24 years	87	19.9	117 593	35.5	1:135[illegible]	0.074
25–29 years	96	21.9	108 746	32.9	1:1133	0.088
30–34 years	72	16.4	49 487	14.9	1:687	0.145
35–39 years	73	16.7	19 522	5.9	1:[illegible]67	0.374
40–44 years	73	16.7	4 880	1.5	1:67	1.496
Above 45 years	19	4.3	306	0.1	1:16	6.209
Total	438	100.0	330 859	100.0	1:755	0.13

30 years of age Ten years earlier the median age of childbearing women was 27 years and 66% of the mothers were below 30 years of age

DISCUSSION

The total incidence of Down s syndrome in Sweden during the 3-year period 1968–70 was 1.37 per mille (1 755) The variation in the incidence during the three years may be random (χ^2=2.6 for 2 d f N S) The recorded incidence is lower than that usually reported, 1.5 per mille (1 650) cf Carter MacCarthy (?) Penrose (12) Hall (5) This may be partly due to underreporting partly —and perhaps more probably—to a real lowering of the incidence owing to the gradual shift to younger childbearing age Characteristic of the Swedish material is the shift to lower age of mothers of children with Down s syndrome compared with earlier reports There has been a continuous decrease in the median maternal age at childbirth during the twentieth century This decrease has been more marked during the last decade (17) the median age of childbearing Swedish women having fallen in the last 10 years from 27 to 25 The significance of such age changes has been pointed out by among others Richards (15) who on the basis of the population figures for England and Wales calculated a decline in the incidence of Down s syndrome from 1 620 in 1939 to 1 795 in 1964 In the present survey 21% of the

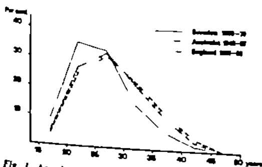


Fig 1 Age distribution of mothers of all live-born children.

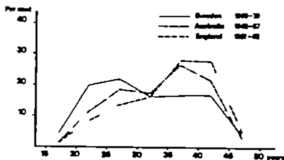


Fig Age distribution of mothers of children with Down s syndrom

mothers were above 40 years of age whereas Penrose in 1954 reported 40% in this age group The effect of age on the incidence may be exemplified by the risk figures for the youngest and oldest maternal groups (below 19 and above 45 years) which in the Swedish material were 1 1685 and 1 16 respectively—a more than hundredfold increase in risk.

A meaningful comparison between incidences of Down s syndrome must therefore take into account differences in maternal age As there is no previous Swedish material which is directly comparable with the present one two foreign materials a British and an Australian have been chosen The former is a pooled British material comprising 2605 cases of Down s syndrome (13) the Australian comprises 1119 cases (3) The British was collected during the period 1951–63 when there were about 1 7 million births the Australian during the period 1942–57 based on about 780000 births The Australian study in particular was characterized by a very intensive attempt to discover the patients

A comparison of the ages of the mothers in the various materials is presented in Fig. 1 The shift towards lower maternal age in the Swedish material compared with the Australian and British is clearly apparent This is also reflected in the distribution of the children with Down s syndrome in relation to maternal age (Fig. 2) The well known bimodal distribution is clearly ap-

Table 1. *Number of Mb Down children born in Sweden 1968–70*

	Boys	Girls	Total	Total number of live-born children	Incidence
1968	94	71	165	113087	1:685
1969	70	61	131	107662	1:821
1970	78	64	142	110150	1:776
Total	242 55.3%	196 44.7%	438	330859	1:755

affect the incidence both of Down's syndrome and of other conditions caused by chromosome aberrations and gene mutations. The aim of the present work was to obtain an up-to-date and as exact as possible survey of the incidence of Down's syndrome in Sweden for comparison with previous materials. At the request of the Swedish National Board of Health and Welfare an inventory of the incidence of Down's syndrome in 1968–70 has been made. Since January 1st 1971 it has been obligatory that cases of Down's syndrome shall be included among those conditions to be reported to the Swedish register of congenital malformations of the National Board of Health and Welfare. Thus, since that date a more careful supervision has been maintained by means of this register.

MATERIAL AND METHODS

The investigation was carried out in the spring of 1971 when records of children with the diagnosis of Morbus Down born in the years 1968, 1969 and 1970 were requested from all pediatric and gynecological departments, all boards for provisions and services to the mentally retarded and all cytogenetic laboratories in the country. The cytogenetic laboratories contributed 25% of the cases which had not been recorded via the other channels. This contribution was evenly spread throughout the maternal age-classes, with one exception—the age group above 45 years, in which only 16% new cases were added. Only cases with a positive chromosome analysis or with an unambiguous clinical picture were included. Of 473 collected cases 35 were excluded owing to a false diagnosis or incomplete documentation of the diagnosis. Data on the total number of children born in Sweden during the same period were obtained from the Central Bureau of Statistics.

RESULTS

The number of liveborn children with Down's syndrome reported in the years 1968, 1969 and 1970 was 438. 83.3% (365) were cytogenetically verified. Of these 365 patients 336 (92.1%) had trisomy 21, 10 (2.7%) an unbalanced D/G and 8 (2.2%) an unbalanced G/G translocation, while 11 (3%) demonstrated a 46/47, +21 mosaicism. The distributions of the cases according to year, sex and incidence for the whole material are shown in Table 1. Table 2 shows the distribution of all the liveborn children with Down's syndrome according to maternal age. At the date of birth of the child 46% of the mothers were below 30 years of age and 21% above 40 years, the median age being 31 years. During the same period the median age of childbearing Swedish women was 25 years and 78% of the mothers were below

Table 2. *Risk of birth of Mb Down children in different age groups 1968–70*

	Mongoloid children		Live-born			
Mother's age	Number *n*	%	*n*	% of all births	Incidence	%
Below 19 years	18	4.1	30325	9.2	1:1685	0.059
20–24 years	87	19.9	117593	35.5	1:135	0.074
25–29 years	96	21.9	108746	32.9	1:1133	0.088
30–34 years	72	16.4	49487	14.9	1:687	0.145
35–39 years	73	16.7	19522	5.9	1:267	0.374
40–44 years	73	16.7	4880	1.5	1:67	1.496
Above 45 years	19	4.3	306	0.1	1:16	6.209
Total	438	100.0	330859	100.0	1:755	0.132

Table 4 *Comparison between numbers of Mb Down children found and expected in the three materials compared in Table 3*

Number found = F number expected at same identification intensity = *E* number expected after correction for different reporting intensity (irrespective of age) = E_{corr} χ^2 calculated on basis of E_{corr}

Age group	Sweden *F*	*E*	E_{corr}	U K. *F*	*E*	E_{corr}	Australia *F*	*E*	E_{corr}	χ^2
–19	18	14 6	17 6	32	33.3	31 0	15	17 1	17.6	0 28
20–24	87	67 3	82.4	220	238 4	235.5	128	119.3	115.8	2.56
25–29	96	80.5	99 8	360	396 0	380.3	208	187.5	184.3	4.28
30–34	77	56.5	70.7	422	436 0	422.9	194	195 6	194.3	0 03
35–39	73	68 0	85 9	731	734	718 6	297	298.8	299 4	.17
40–44	73	53 9	68 0	708	701.2	686.0	240	265 9	266.3	3.67
45–	19	8 7	10.9	132	134	130.5	37	45 1	45 0	7 46**
Total	438	349.5		2605	2683 3		1119	1129 3		20.45

Each χ^2 within an age group is based on 2 d f and the total accordingly on 14 d f

** $0\ 025 > p > 0\ 01$

den (87+220×1.29+128×1 26) 532.1 cases should have been recorded in this age group

The British material includes 432 500 births in this age group of a total of 759 300 for the three materials Accordingly for the same incidence in all three materials the number of children with Down's syndrome expected to be reported would then have been 532 1× 432 500/759 300 Due to the underreporting the expected number has to be corrected 303.8 1 29=235 5 The observed number was 220 children The E_{corr} values calculated in this way are shown in Table 4 On the basis of these expected values the heterogeneity between the materials can be tested both within age groups and in the total material These calculations form the basis for the figures in Table 4 from which it is apparent that discrepancies between the materials *can* be explained by a higher ascertainment factor in the Swedish material It should be pointed out however that in the highest age group there is an increase which in itself is significant. If this increase is random it would imply that the correction introduced for a higher degree of identification does not entirely compensate for the rise in this age group As it is open however and is characterized by a steeply rising risk within the age group even small changes of the distribution within the age group may explain such differences Of the 19 Swedish mothers in this group nine were 45 seven 46 one 47 and two 48 years of age Penrose (13) gives 46 years as the central point in the age groups above 45 years in his material

Even if a higher degree of identification intensity is the most probable reason for the higher Swedish incidences in the various maternal age groups one cannot yet entirely disregard the possibility that there has been a real increase of the incidence of Down's syndrome in 1968–70 A combination of the two explanations may of course also exist

ACKNOWLEDGEMENT

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Table 3 *Comparison between the Swedish figures with those published by Penrose (1965) from the U K and Collman & Stoller (1962) from Australia*

	Sweden 1968–70 (331 000 births)			U K 1951–63 (1 700 000 births)			Australia 194 –57 (780 000 births)				
Age group	Mb Down	% of all births	% risk	Mb Down	% of all births	% risk	Mb Down	% of all births	% risk	χ^2_{ea}	χ^2
–19	18	9 2	0 59	3?	4 07	0 45	15	4.56	0 4?	0 1	1 1
20– 4	87	35.5	0 74	220	_5.5	0 49	128	26 7	0 61	3 0	9 6
25–29	96	32.9	0 88	360	31 5	0 65	20?	32.5	0.82	5.3**	8.5 **
30–34	7?	14 9	1 46	4_2	22 4	1 08	194	?1 9	1 13	0 1	4 7
35–39	73	5 9	3 74	731	17 4	3.37	?97	11 0	3 45	0 1	0 4
40–44	73	1 5	14 96	708	3 8	10 74	?40	3 14	9 80	2.2	9.3*
45–	19	0 1	62 10	13?	0 3	4 94	37	0 22	?1.56	1 0	13 7*
Total	438			2605			1119			11 6	47.3 **

Each χ^2_{ea} is based in 1 d.f and the total χ^2 accordingly on 7 d.f Each χ^2_t is based on 2 d.f and the total on 14 d.f

** 0 025>p>0 01.
0 01>p>0 001
p<0 001.

parent in the Australian and British materials with a minor peak between 25 and 29 years of age (non age-dependent Down syndrome) and a later pronounced peak between 35 and 44 years of age (age-dependent Down syndrome) In the Swedish material the picture is reversed The risk figures for each maternal age group in the three materials are shown in Table 3 Within each age group a comparison has been made between the British and Australian material (χ^2ea) as well as between all the three materials ($\chi^2 t$)

The incidences in the various maternal age groups are fairly similar in the British and Australian materials and the differences may very well be random (χ^2=11 6 for 7 d f 0 2>p>0 1) The Swedish material has a higher incidence in all age groups than either of the other materials If each age group is analysed separately a significant difference is found between the three materials in the 20–24 and 25–29 age groups and in the two groups above 40 years of age

Different explanations for the differences in maternal age are conceivable One possibility is that the Swedish material is more complete owing to more effective confirmation of cases This hypothesis can be tested if the confirmation is considered to be independent of maternal age (Table 4) In the Swedish material altogether 438 children with Down s syndrome have been identified With an identical risk within each age group for all the materials pooled a total of 349 5 Down children would have been expected if the confirmation efficiency was the same The ratio between the values found and expected is 1 25 which constitutes an estimate of the ascertainment efficiency In the same way the ratio for the British material is 0 97 and for the Australian 0 99 Therefore the British cases would be underidentified by a factor of 1 25 0 97=1 29 and the Australian cases by a factor of 1 25 0 99=1 26 compared with the Swedish material One can then calculate the expected true number of Down children in the British and Australian materials—i e at the same reporting intensity as in the Swedish material—and then by dividing these numbers by 1 29 and 1 26 respectively calculate the expected identified numbers at the same incidence in all materials within each age group

The mathematical expectation (E_{corr} in Table 4) for the age group 20–24 years in the British material is for example calculated as follows If all three materials had been reported with the same intensity as in Swe

Table 4 *Comparison between numbers of Mb Down children found and expected in the three materials compared in Table 3*

Number found = F number expected at same identification intensity = E number expected after correction for different reporting intensity (irrespective of age) = E_{corr} χ^2 calculated on basis of E_{corr}

Age group	Sweden F	Sweden E	Sweden E_{corr}	U K F	U K E	U K E_{corr}	Australia F	Australia E	Australia E_{corr}	χ^2
19	18	14 6	17 6	32	33.3	31 0	15	17 1	17 6	0.28
20– 4	87	67.3	82.4	220	48 4	235 5	128	119 3	115.8	2.56
25–29	96	80.5	99 8	360	396 0	380.3	208	187.5	184.3	4.28
30–34	77	56.5	70.7	422	436 0	422 9	194	195 6	194.3	0 03
35–39	73	66.0	85 9	731	734 2	718 6	297	298.8	299 4	..17
40–44	73	53 9	68.0	708	701	686.0	40	265 9	266.3	3.67
45–	19	8.7	10.9	132	134..	130 5	37	45 1	45 0	7 46**
Total	438	349.5		2605	683.3		1119	1179 3		20.45

Each χ^2 within an age group is based on d.f and the total accordingly on 14 d.f

** $0.025 > p > 0.01$

den ($87+220\times1.29+128\times1\ 26$) 532.1 cases should have been recorded in this age group

The British material includes 432 500 births in this age group of a total of 759 300 for the three materials. Accordingly for the same incidence in all three materials the number of children with Down s syndrome expected to be reported would then have been 532 1× 432 500/759 300 Due to the underreporting the expected number has to be corrected 303.8 1.29=235.5 The observed number was 220 children. The E_{corr} values calculated in this way are shown in Table 4 On the basis of these expected values the heterogeneity between the materials can be tested both within age groups and in the total material These calculations form the basis for the figures in Table 4 from which it is apparent that discrepancies between the materials *can* be explained by a higher ascertainment factor in the *Swedish material It should be* pointed out however that in the highest age group there is *an increase* which in itself is significant If this increase is random, it would imply that *the correction introduced* for a higher degree of identification does not entirely compensate for the *rise in this* age group As it is open however and is characterized by a steeply *rising risk* within the age group even small changes of the distribution within the age group *may explain* such differences Of the 19 Swedish mothers in this group nine were 45 seven 46 one 47 and two 48 years of age Penrose (13) gives 46 years as the central point in the age groups above 45 years in his material

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THE RELATION OF SIZE AT BIRTH AND PRESCHOOL CLINICAL SEVERE MALNUTRITION

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ABSTRACT Cravioto, J and DeLicardie, E. R. (Scientific Research Division, Hospital del Niño IMAN, México, D. F México). The relation of size at birth and preschool clinical severe malnutrition. Acta Paediatr Scand 63: 577 1974.—Examination of the relationship between infant's size at birth and occurrence of postnatal severe malnutrition is important because if size at birth predicts the development of malnutrition in childhood identification of children at risk and prevention could be carried on more efficiently The opportunity for such an analysis presented itself when in the course of a longitudinal study beginning at birth of 334 infants, 22 developed severe malnutrition, despite the fact that all children were examined on a biweekly basis, growth failures identified, infectious illness treated, and the parents given advice (which they did not follow) on the appropriate feeding and care of the child. Mean weight, length, head, chest and arm circumferences and skinfold thickness at birth were almost identical between the group of index cases and the whole birth cohort. Variances in each of these measures were also equivalent. Levels of association among growth measures were not significantly different. No association obtained between size at birth and age at which severe malnutrition occurred. Neither true prematures nor small-for-date infants were overrepresented in the malnourished group. The hypothesis that infants with presumably higher nutritional requirements are at greatest risk of developing severe malnutrition is not sustained by the data.

KEY WORDS: Size at birth, postnatal malnutrition

There has been a tendency to consider (4) that in situations in which food provision for infants and young children is marginal intrinsically larger infants would presumably have higher nutritional requirements and are therefore at greatest risk for the development of severe malnutrition. From the public health point of view an analysis of the relation between size at birth and the subsequent occurrence of severe malnutrition in early childhood is important because if size at birth helps to define the likelihood with which a child would develop severe malnutrition during infancy and the preschool years such knowledge would be valuable for the identification of children at risk and potentially for prevention

In the course of a longitudinal study beginning at birth 22 of the 334 infants being followed-up developed severe clinical malnutrition by 4 years of age Such cases occurred despite the fact that all children in the cohort received pediatric examinations

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on a biweekly basis growth failures were identified infectious and other illness diagnosed and the parents given detailed advice on the appropriate feeding and management of the child (2) The occurrence of severe malnutrition under such circumstances makes possible an anterospective analysis of the etiology of severe malnutrition Such an analysis obviously has many aspects both biologic and social In the present report however we restrict our consideration to an examination of the relationship between the size of the child at birth and the occurrence of servere malnutrition during the first 4 years of life

SUBJECTS AND PROCEDURE

The overall design of our anterospective longitudinal study has been described in detail elsewhere (2) and needs only to be briefly summarized. The study is an ecologic one in which a total cohort of 13 months of births ($N=334$) is being followed from birth through the first school years Children are examined by pediatricians biweekly and at that time are also weighed and measured At specified times behavioral and familial evaluations are carried out The setting is a rural village of approximately 6 000 people in Southwest Mexico and the data are gathered through predefined protocols by a resident team of pediatricians psychologists social workers, nurses and nutritionists.

Of all the children in the birth cohort 78 % were born in their own homes under the care in almost all cases of a trained semiprofessional local midwife These practitioners either did not weigh the child at birth or if they did so used uncalibrated scales the reliability and validity of which were highly questionable It was therefore necessary for birth weight to be obtained by one of the pediatricians of the field team using recently calibrated equipment At the time of weighing a physical examination of the child was performed anomalies were noted, and careful measurements were made of total body length head circumference chest circumference arm circumference and skin fold thickness. In the great majority of cases these observations occurred on the day of birth In the remainder in most instances, on the second day In a few cases first weighings were delayed by as much as 48-72 hours. Because it was possible that these delayed measurements could systematically decrease the magnitude of the birth weight estimate 100 children who had been weighed on the first day of life were reweighed on the third day No overall systematic decrement was noted at reweighing, so that it was most unlikely that the small number of late weighings resulted in a systematic reduction in the estimation of birth weight The absence of weight loss during the first days of life is probably related to the practice of giving tea to the infant as early as 6 hours after delivery

Nine of the 334 infants were considered as true prematures since their gestations lasted between 31 and 35 weeks Two of these 9 infants were small-for-dates (birth weights of 1255 g and 1640 g) All the other infants delivered within the calendar year had gestational ages between 36 and 42 weeks

In regard to the distribution of birth weights in the total annual cohort there were 2.7 % of infants weighing 1 500–1999 g 9 6 % weighing 2 000–2499 g; 50.5 % weighing 2 500–2 999 g 29 6 % weighing 3 000–3 499 g, and 7 6 % weighing 3 500–3 999 g. Mean birth weight was 2 898 ± 444 g. A birth weight of less than 2 500 g was thus obtained for 12 4 % of the cohort, whereas only 7 6 % of the children weighed more than 3 500 g The mean birth weight of boys was significantly higher than that of girls. The mean birth weight of boys was 2977 ± 394 g and that of girls 2860 ± 408 g The mean difference of 117 g was significant at the 0.02 level of confidence

The mean birth weight of these children is significantly below that of Swedish and North American infants (3) It closely resembles the mean birth weight of Indian infants in Delhi Negro infants of the French Sudan and Indian infants from Singapore (1)

The median body length was 48.5 cm, with 25 % of the children having a body length of less than 47 cm and an equal number having body lengths between 49.5 and 53 cm As was the case for birth weight mean body length at birth was significantly higher in boys than in girls. Boys had a mean length value of 48 7 ± 1 8 cm and girls one of 48 0 ± 2 0 cm This difference though small absolutely is statistically significant ($t=3\ 3$ $p < \ 01$)

The severely malnourished children

As stated above 22 cases, 14 girls and 8 boys, were diagnosed as suffering from clinical severe malnutrition Age at the time of the diagnoses ranged from 4 to 53 months with a single infant below 1 year of age 9 cases between 1 and years 8 cases between and 3 years of age 3 patients with ages between 3 and 4 years, and 1 case diagnosed at 53 months of age One of these patients was born at a gestational age of 34 weeks weighing 2 820 g All the other cases were full-term infants.

Fifteen of the 22 patients corresponded to the kwashiorkor type the other 7 cases were of the marasmic variety The proportion of marasmus in females and males was 4 3 while the number of females with kwashiorkor was twice the number for boys Probably due to the small number of cases these differences are not statistically significant at the level of confidence of 0 05

RESULTS

As may be seen in Table 1 mean weight height head circumference chest circumference arm circumference and skinfold

ble 1 *Size at birth in index cases and cohort a whole (Land of the White Dust)*

	Index cases		Cohort			
	Mean	S. D	Mean	S D	*t*	*p*
eight g)	2 855	417	898	444	0 45	N S.
eight cm)	48 0	~1	48.3	1 9	0 63	N.S
ead circumference cm)	33 9	1 0	33 7	1 4	0.61	N.S.
hest circumference cm)	31 9	1 7	32.2	1.8	0.70	N.S
rm circumference (cm)	9 9	1 1	10 0	0.9	0.44	N.S
kinfold thickness (cm)	4.3	1.3	4 4	1 0	0.38	N.S.

hickness at birth are almost identical between the index cases as a group and the otal birth cohort. Variances in each of these measures are also equivalent between groups and *no difference in mean values is statisti*cally significant.

Because it was possible that despite an absence of difference in growth attainment interrelations among growth measures at birth could be different the index group and the cohort were compared with respect to the correlations among growth measures that were present All correlations were positive and significant indicating a clear association among measures in both groups (Table 2) The groups did not differ significantly in the levels at which such associations occurred.

Since sex differences were possible sex specific comparisons were made between the *index cases and the cohorts both for mean* values and for the association among measures The findings for each sex were replicates of those obtained for the group as a whole

If size at birth were an important factor in determining vulnerability to severe malnutrition it would be most likely to be reflected in those children who develop malnutrition *early in life One would therefore* from this point of view expect to find a significant relation between growth achievement at birth and severe malnutrition in children who develop the syndrome early and less likely to find it in children who become severely malnourished at later ages For this reason we have explored birth size in relation to the age at which severe clinical malnutrition occurred This analysis *indicates no systematic* relation between birth size and age of incidence of severe clinical malnutrition.

Table 2. *Intercorrelations among growth measures in index cases and cohort (Land of the White Dust)*

	Weight		Height		Head circ.		Chest circ		Arm circ.		Skinfold	
Weight	–	–	0.54	C	0 39	C	0.86	C	0 82	C	0.65	C
	–	–	I	0 73	I	0.71	I	0.82	I	0.73	I	0.44
Height					0.49	C	0 49	C	0.37	C	0 27	C
					I	0 70	I	0 63	I	0 51	I	0.29
Head circ							0 61	C	0.46	C	0.32	C
							I	0 63	I	0 52	I	0.27
Chest circ									0 76	C	0.69	C
									I	0 64	I	0.40
Arm circ											0 78	C
											I	0.50
Skinfold												

I = Index cases

DISCUSSION

Our findings indicate no systematic relations between size at birth and the development of severe clinical malnutrition in infancy and early childhood Moreover no systematic differences in size at birth are associated with the age at which such severe malnutrition occurs

At the practical level such data suggests that size at birth cannot be used as a good predictor of risk for the subsequent development of severe clinical malnutrition They lead to the hypothesis that the factors which are influencing intrauterine growth may be quite different from those which influence nutrition and growth in the postnatal period

At the theoretical level the findings provide no support either for the hypothesis that larger children are at greater risk for severe malnutrition or that small size at birth is continuous with the processes contributing to the development of severe malnutrition in infancy and early childhood Thus size at birth is unrelated to the occurrence of severe clinical malnutrition in the postnatal period

It should not be assumed from these findings that perinatal conditions other than size are unrelated to the subsequent development of severe malnutrition Behavioral attributes of the child neurologic abnormalities as well as peculiarities of the mother-infant interaction may all be relevant predictors of disturbed nutritional outcomes and the relation of such factors to severe malnutrition will be considered in subsequent reports However the data of the present report strongly indicate the absence of a relation between size at birth and severe clinical malnutrition in infancy and early childhood.

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ANAEROBIC BACTERIA AND DECONJUGATED BILE SALTS IN THE UPPER SMALL INTESTINE OF INFANTS WITH GASTROINTESTINAL DISORDERS

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ABSTRACT Challacombe, D. N., Richardson, J M. and Edkins, S. (Institute of Child Health University of Birmingham, Birmingham, England). Anaerobic bacteria and deconjugated bile salts in the upper small intestine of infants with gastrointestinal disorders. Acta Paediat Scand, 63: 581 1974.—Deconjugated bile salts have been reported in the upper small intestine of infants with protracted diarrhoea and secondary monosaccharide intolerance. As deconjugated bile salts inhibit monosaccharide transport mechanisms in the small intestine of experimental animals both in vivo and in vitro, they may also cause monosaccharide malabsorption in these infants. In this study infants and children with protracted diarrhoea have been challenged with oral sugar loads to detect patients with glucose or lactose intolerance. The duodenal juice of all infants with protracted diarrhoea was examined by thin layer chromatography and fluorimetry for deconjugated bile salts and cultured anaerobically for bacteria with known deconjugating properties. In addition duodenal juice from infants and children with other gastrointestinal disorders was similarly studied. Deconjugated bile salts and obligate anaerobic bacteria were only associated in two out of thirty samples of duodenal juice, one from an infant with secondary lactose intolerance, and one from an infant with unexplained failure to gain weight. The absence of deconjugated bile salts in the duodenum of two infants with secondary monosaccharide intolerance and from other infants with protracted diarrhoea, does not support the suggestion that the action of deconjugated bile salts on the small intestinal mucosa, is an important causative factor in these disorders.

KEY WORDS: Protracted diarrhoea, secondary monosaccharide intolerance, deconjugated bile salts, anaerobic bacteria

The upper small intestine of infants without diarrhoeal disorders may be sterile or contain micro-organisms which are similar in type to those isolated from the stomach throat or nose (3) The upper small intestine of infants with protracted diarrhoea and sugar intolerance either following an acute attack of diarrhoea or small intestinal surgery is rarely sterile (4 6 11) The duodenal microflora in these infants is characterised by an increase in numbers and types of organisms and by the presence of coliforms particularly *Escherichia coli* (4) The degree of abnormality of the aerobic microflora in these reports (6 11) was shown to parallel the severity of sugar intolerance but culture of anaerobic organisms was not attempted

Deconjugated bile salts have been demonstrated in the small intestine of some patients with protracted diarrhoea and secondary monosaccharide intolerance (11) As anaerobic bacteria may deconjugate bile salts in vitro (15) it was suggested that the presence of deconjugated bile salts in these patients may have been the result of an undetected anaerobic microflora (11)

Deconjugated bile salts have also been shown to impair small intestinal monosac

Table 1 *Details of infants and children studied*

Patient no	Age	Sex	Diagnosis
Group 1 Infants with Chronic Diarrhoea			
1	6 weeks	M	Chronic non-specific gastroenteritis
2	2 months	F	Chronic non-specific gastroenteritis
3	3 months	F	Chronic non-specific gastroenteritis
4	4 months	F	Chronic non-specific gastroenteritis
5	1 month	F	Secondary monosaccharide intolerance
6	3 months	F	Secondary monosaccharide intolerance
7	4 months	M	Secondary lactose intolerance
Group 2 Post surgical infants			
8	1 month	M	Gastro-colic fistula
9	2 months	F	Congenital jejunal strictures
10	3 months	F	Duodenal atresia
11	4 months	M	Hirschsprungs disease
12	1 year	F	Hirschsprungs disease
13	1 year 6 months	M	Hirschsprungs disease
14	5 years	M	Hirschsprungs disease
Group 3 Other gastrointestinal disorders			
15	9 months	F	Coeliac disease
16	1 year	F	Coeliac disease
17	1 year 6 months	F	Coeliac disease
18	1 year 10 months	F	Coeliac disease
19	2 years 2 months	F	Coeliac disease
20	3 years	F	Coeliac disease
21	4 years	M	Coeliac disease
22	7 years	F	Coeliac disease
23	3 months	F	Unexplained failure to gain weight
24	11 months	M	Unexplained failure to gain weight
25	3 years	F	Unexplained failure to gain weight
26	4 years	M	Unexplained failure to gain weight
27	5 years	M	Unexplained failure to gain weight
28	1 year 9 months	F	Cystic fibrosis
29	2 years	M	Hypogammaglobulinaemia
30	8 years	F	Ileal blind loop

charide transport mechanisms in experimental animals both in vitro (12 20) and in vivo (13) As bile salts in the duodenum of normal infants are usually conjugated bacterial deconjugation of bile salts in the small intestine of infants with protracted diarrhoea and secondary monosaccharide intolerance might be closely related to the development of a monosaccharide transport defect (11)

The relationship between an anaerobic microflora and deconjugated bile salts in the upper small intestine of infants and children with protracted diarrhoea, and with other gastrointestinal disorders was therefore studied Some of these patients were intolerant of dietary sugars

MATERIAL

The infants and children studied were divided into three clinical groups. None had been treated with antibiotics for two weeks prior to intubation nor had known enteropathogenic bacteria been isolated from the faeces. Details of the patients studied are shown in Table 1

Group 1 Infants with Chronic Diarrhoea

These infants presented with an acute attack of diarrhoea which did not respond to oral or intravenous rehydration Chronic Diarrhoea in this investigation is defined as the passage of four or more loose watery stools a day for a period in excess of two weeks. This group consisted of four infants with chronic nonspecific gastroenteritis two infants with secondary monosaccharide (glucose) intolerance and one infant with secondary lactose intolerance The infants with glucose and lactose intolerance had been able to tolerate these dietary sugars before the diarrhoeal illness.

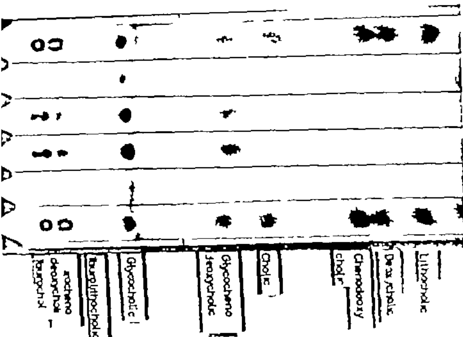


Fig. 1. The thin layer chromatographic method used in this investigation. The two outer tracks were loaded with 20 micrograms of a standard mixture of bile salts (Maybridge Research Chemicals, Launceston, Cornwall) while the four inner tracks were loaded with 10 microlitres of duodenal juice.

Group 2. Post-surgical infant

This group of infants and children presented with failure to gain weight and protracted diarrhoea, following partial resection of the large or small intestine.

Group 3. Other gastrointestinal disorders

This group consisted of 8 patients with untreated coeliac disease (flat villi on small intestinal biopsy and subsequent weight gain following gluten withdrawal), 5 patients with unexplained failure to gain weight and single cases of cystic fibrosis, hypogammaglobulinaemia, and ileal blind loop.

METHODS

1. *Sampling and microbiological culture techniques*

As variations in the duodenal microflora may occur during the day and especially after a meal (9), the following sampling regime was devised in relation to feeding in all patients. A milk feed was given at 8.00 a.m. via the feeding catheter (Argyl 5FG, 91 cm in length, weighted with a gold bead) (21), was inserted into the stomach at 9.00 a.m. and the duodenal sample taken at 10.00 a.m., 2 hours after the milk feed. The position of the gold bead in the 3rd–4th part of the duodenum was confirmed by screening.

One in 20 dilutions of duodenal juice in transport medium were delivered promptly to the laboratory. Within 2 hours, aliquots of 0.5 ml were removed and serial tenfold dilutions made of each aliquot, in glucose broth. Anaerobic organisms were cultured on blood agar in a modified McIntosh and Fildes jar (Baird and Tatlock, London) using the method described by Drasar (8). The presence of *Bacteroides* sp. was confirmed by gram stain and subculture. Nonsporing, gram negative strictly anaerobic rods were classified as *Bacteroides* sp. The quantitative results of bacterial culture were expressed as the $\log_{10}$ of the viable organism count/ml of intestinal juice. Thus 5000 organisms/ml = 3.7 $\log_{10}$ *viable organisms/ml of juice.*

2. *Bile salt estimation*

Bile salts were estimated qualitatively and quantitatively by thin layer chromatography and fluorimetry (19). This method gave good resolution of both conjugated and deconjugated bile salts but did not separate the individual taurine or glycine conjugates of dihydroxy bile salts (Fig. 1). Each sample of duodenal juice was divided and used for bile salt estimations and for bacterial culture.

3. *The diagnosis of sugar intolerance*

The stools of all infants in Groups 1 and 2 were collected in a plastic square inserted inside the nappy or into a urine bag with the opening enlarged and placed over the anus. Each patient was fed a 5% lactose solution (2 g lactose/kg body weight) and the stools were collected over the following 4 hours and tested with

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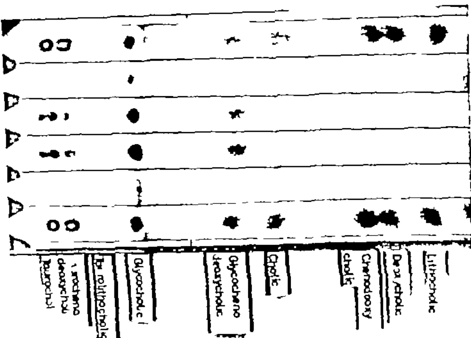


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Table 2 *Patients growing obligate anaerobes with bile salt deconjugating properties from their duodenum*

Viable organism count $\log_{10}$/ml of duodenal juice

Patient no	Age	Diagnosis	Anaerobic lactobacilli	Bacteroides sp	Veillonella	Clostridia
5	1 mo	Secondary monosaccharide intolerance		5		
7	4 mo	Secondary lactose intolerance		6		
15	9 mo	Coeliac disease				3.3
20	3 y	Coeliac disease		5 3	5 7	
21	4 y	Coeliac disease		4 9		
24	11 mo	Unexplained failure to gain weight	6	4.3		
25	3 y	Unexplained failure to gain weight			5.3	
29	[illegible] y	Hypogammaglobulinaemia		2		

Clinitest tablets (Ames Co) (17). A colour reaction of 1/2% or more was taken to indicate intolerance of the lactose load The following day a feed of 5% glucose solution was given (2 g glucose/kg body weight) the stools were tested with Clinitest and the results interpreted as before Infants with lactose or glucose intolerance usually responded rapidly to the sugar challenge with profuse watery diarrhoea. In malnourished and dehydrated infants the sugar load was not given unless an intravenous drip of 1/5 normal saline was set up prior to the challenge to prevent further dehydration

RESULTS

In only three infants all in Group 1 were significant levels (more than 1/2%) of reducing substances detected in the stools

Duodenal microflora

Although aerobic and anaerobic organisms were isolated from the duodenum only the anaerobic organisms are reported in this communication Culture of the aerobic microflora is reported elsewhere (4) Obligate anaerobes with known deconjugating properties (15) were isolated from the duodenal juice of patients in Groups 1 and 3 only (Table 2) In Group 1 *Bacteroides* sp were isolated from the duodenum of one infant with secondary lactose intolerance and from another with secondary monosaccharide intolerance In Group 3 obligate anaerobes were isolated from the duodenum of two infants who were failing to gain weight three patients with untreated coeliac disease and one child with hypogammaglobulinaemia. None of the post surgical infants with protracted diarrhoea grew anaerobes from the duodenum Anaerobes were therefore found in 8 of the 30 infants and children studied in this investigation

Bile salts

Deconjugated bile salts were found in the duodenal juice of only three patients (Table 3) In the first (Number 7) an infant with secondary lactose intolerance the deconjugated bile salts cholate chenodeoxycholate and lithocholate were found in the duodenal juice and obligate anaerobes *Bacteroides* sp were cultured In the second an infant with unexplained failure to gain weight (Number 24) chenodeoxycholate was found in the duodenal juice in association with Anaerobic Lactobacilli and *Bacteroides* sp In the first of these patients the total concentration of deconjugated bile salts exceeded 50% of the total concentration of bile salts In the duodenal juice of a third patient (Number 30) a child with an ileal blind loop following side to-side ileo-ileal anastomosis for ileal atresia in infancy (5) chenodeoxycholate and lithocholate were demonstrated in the duodenal juice in the presence of staphylococci These organisms are facultative anaerobes and are sometimes capable of deconjugating bile salts in vitro

Table 3 *Patients with deconjugated duodenal bile salts (mM/l of duodenal juice)*

Patient no	Age	Diagnosis	Total bile salts	Deconjugated bile salts		
				Cholate	Chenodeoxy cholate	Litho-cholate
7	4 mo	Secondary lactose intolerance	[illegible]	0.13	0.57	0.25
4	11 mo.	Unexplained failure to gain weight	1.20	Not detected	0.27	Not detected
[illegible]	8 y	Ileal blind loop	6 60	Not detected	0 40	0 79

(15) Of the 8 patients from whom obligate anaerobes were isolated deconjugated bile salts were absent in the duodenum in 6 In the three patients with deconjugated bile salts in the duodenum chenodeoxycholate was found in all three lithocholate in two and cholate in one

DISCUSSION

Anaerobic microorganisms with deconjugating properties are usually absent from the normal upper small intestine The low oxidation-reduction potential and intestinal stasis necessary for their growth only occurs in the terminal ileum and large bowel However in gastrointestinal disorders causing stasis such as blind loops strictures or diverticulae these organisms may proliferate within the small intestine (24) Obligate anaerobes together with deconjugated bile salts have been demonstrated particularly in those areas of the small bowel where stagnation of intestinal contents occurred (9 10)

In this investigation obligate anaerobes were present in the duodenum in 8 out of 30 infants with gastrointestinal disorders However deconjugated bile salts and anaerobes were only found to coexist in 2 infants suggesting that additional factors such as small intestinal stasis may be necessary before deconjugation of bile salts occurs in the duodenum The absence of deconjugated bile salts in 6 patients from whom obligate anaerobes with deconjugating properties were isolated from the duodenum (Table 2) may either indicate that the degree of small intestinal stasis was insufficient for bile salt deconjugation to occur or that deconjugated bile salts were absorbed in the upper small intestine as rapidly as they were formed (16) It is also possible that a higher incidence of anaerobes and deconjugated bile salts would have been found if sampling had taken place at multiple levels in the upper small intestine

Deconjugated bile salts have been shown to be toxic to the epithelial cells of the small intestine in laboratory animals (18) and to inhibit many metabolic functions of these cells including monosaccharide transport, both in vivo and in vitro (12 13 20) They have also been demonstrated with an abnormal aerobic microflora in the upper small intestine of infants with protracted diarrhoea, and a temporary inability to absorb simple dietary sugars including monosaccharides (11) It was therefore suggested that deconjugated bile salts in the small intestine of these infants might be closely related to the development of a monosaccharide transport defect (11) We were unable to demonstrate deconjugated bile salts in two infants with Chronic Diarrhoea and secondary monosaccharide intolerance but in one of these patients *Bacteroides* sp. (obligate anaerobes with known deconjugating properties) were isolated from the duodenal juice in a concentration of 5 log_{10}/ml.

Deconjugated bile salts were demonstrated in the duodenum of one infant with secondary lactose intolerance The concentration

of deconjugated bile salts in this infant was lower than the concentration reported to impair sugar transport in rats with jejunal blind loops in vivo (14) or to produce toxic changes to epithelial cells of guinea pigs and hamsters in vivo (18) Deconjugated bile salts were also found in the duodenum of two patients who tolerated the sugar challenges normally The first of these was failing to gain weight and the second had an ileal blind loop

We were unable to demonstrate the presence of the deconjugated bile salt deoxycholate in the duodenum of any patient This bile salt has been shown to have cytotoxic and metabolic inhibitory effects on the small intestine of experimental animals at lower concentrations than other deconjugated bile salts However lithocholate a deconjugated monohydroxy bile salt which also has cytotoxic properties was found in the duodenum of two patients

The infrequent association between anaerobes and deconjugated bile salts in the duodenum of infants with Chronic Diarrhoea and sugar intolerance or of infants with Post-surgical diarrhoea does not suggest that this association is an important contributory factor to the aetiology of these disorders As small intestinal stasis and delayed intestinal transit have been reported in infants with acute diarrhoea (22) and in infants with diarrhoea and malnutrition (7) the association of anaerobes and deconjugated bile salts in two of our patients may have been secondary to a motility disorder of the small intestine causing intraluminal stasis

Small intestinal biopsies from some patients with acute or protracted diarrhoea have demonstrated varying degrees of villous damage (1 23) As infants with protracted diarrhoea usually commence their illness with an acute attack of diarrhoea it is possible that certain enteropathogenic organisms may initially damage the small intestinal mucosa causing net fluid secretion and impaired carbohydrate absorpt Both of these factors may continue for : eral weeks before the mucosa recovers persistence of enteropathogenic organis within the lumen of the small intestine co be one factor delaying mucosal recove Disorders of carbohydrate absorption in | tients with protracted diarrhoea may be t direct result of continuing mucosal dama by enteropathogenic organisms

ACKNOWLEDGEMENTS

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LYSOZYMES IN FECES FROM INFANTS AND CHILDREN

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ABSTRACT Haneberg B. and Finne P (Department of Pediatrics and the Broegelmann Research Laboratory for Microbiology The University of Bergen, School of Medicine, Bergen, Norway) Lysozymes in feces from infants and children. Acta Paediatr Scand 63: 588 1974.—An agar diffusion technique seemed useful for the assay of lysozyme activity in serum milk, and extracts of freeze-dried feces. Normal levels of this activity were established before the method was applied to extracts of feces from premature infants receiving either human milk with lysozyme activity of its own, or cow's milk with or without added albumen lysozyme Results of these investigations, in addition to immunologic studies, using antisera to human and albumen lysozymes, indicated that human milk lysozyme and albumen lysozyme in cow s milk formula may pass through the infants intestinal tract. Albumen lysozyme however will most often be inactivated before being excreted with feces. Low fecal pH was found in those receiving albumen lysozyme as well as in those receiving human lysozyme This may reflect the intestinal activity of the lysozymes. Low intestinal pH, brought about by lactulose in the cow's milk feed, did not in itself lead to increased lysozyme activity At present no advantage of adding albumen lysozyme to cow s milk formula has been substantiated

KEY WORDS: Lysozyme feces, infants, children

Lysozymes or muramidases represent a group of enzymes widely distributed in nature (5) Human lysozyme is found in cells serum and secretions (5) including milk (2) and sometimes in feces (1 6) The enzyme is more constantly found in feces from breast fed infants than in feces from infants fed cow s milk (9)

Lysozymes will lyse bacteria through their effect on the cell wall (5) Their action on *Micrococcus lysodeikticus* is most readily demonstrated and is used for assay of the lytic activity (7) *In vitro* pathogenic bacteria will be lysed by the lysozymes only after exposure to heat acid alkali or various chemicals (3 4 14 16) by which the substrate is probably unmasked Antibodies together with complement also seem to render the cell wall accessible to lysozyme (5) Thus lysozymes may play an active role in the defence against bacterial infections

Lysozymes from various animal species differ as revealed by immunological methods although they all act on *M lysodeikticus* (5) Egg white is a rich source of lysozyme and is used for commercial preparation of the enzyme Human milk contains much more lysozyme than does cow s milk (2) Enrichment with albumen[1] lysozyme would therefore render cow s milk more similar to human milk with regard to lysozyme activity Cow s milk formulas for infant feeding, with small amounts of lysozyme added are on the market in some countries Clinical benefits of such formulas are reported (12)

[1] Hen egg white

The present study was undertaken in order to investigate the effect of lysozyme enriched cow's milk formula on the lysozyme activity and the pH of feces from premature infants. Since lactulose in milk is known to lower fecal pH (8) the effect of this on fecal lysozyme activity was also studied.

Normal levels of lysozyme activity in human serum, milk and feces were established by our quantitative method.

MATERIALS AND METHODS

Individuals

Thirty-eight premature infants (birth weights 1040–2140 g) admitted to the Children's Hospital, Bergen, were fed exclusively either human milk, commercial cow's milk formula, or this formula with added lysozyme, for 1 to 4 weeks during their first month of life.

Another 6 infants were given commercial cow's milk formula for some days, thereafter with lactulose added to the formula.

Ninety-five healthy infants and children were studied as controls to establish normal levels of lysozyme activity in serum and feces. None of these infants received human milk. The lysozyme activity in human milk from 37 healthy lactating women were determined at various stages after parturition.

Fecal extracts

Feces from the premature infants, fed any one of the three kinds of milk for at least 1 week, were collected twice weekly for 24 hours. From the healthy controls, samples of one defecation were taken. The fecal samples were stored at −20°C until processed. As outlined before (10), feces negative for blood by benzidine test, were freeze-dried, and extracts were made by suspending 1 g of the dry fecal matter in 10 ml phosphate-buffered saline (PBS), pH 7.2, after which the suspension was centrifuged at 20 000 g for 30 minutes at 4°C. The supernatant, called extract, was pipetted off and stored at −20°C until used.

Milk

Human milk was collected by the use of breast pump and stored at 4°C for up to 24 hours before giving it by bottle or gastric tube. Samples of the individual 24-hour-volumes of milk were stored at −20°C.

Ordinary infant formula (Collett A/S, Asker, Norway) was based on dried cow's milk. Lysozyme-enriched cow's milk was made from this same infant formula with albumen lysozyme (Eisai Co., Ltd, Tokyo, Japan) added while in dry state to make up a final concentration of 400 μg/ml. Lactulose was also added to part of the regular infant formula to a concentration of 8 mg/ml. The infant formulas were stored in a dry state at room temperature, and mixed with preheated water the day given to the babies.

Before testing, fat was removed from human milk and cow's milk formulas by centrifugation at 20 000 g for 20 minutes at 4°C.

Sera

Serum was obtained from the infants and children at the end of the feeding experiment or at the time when feces were collected. Rabbit antiserum to albumen lysozyme was kindly supplied by Dr A. Grov, The Gade Institute, Department of Microbiology, University of Bergen. Goat antiserum to human lysozyme was purchased (Miles Laboratories Inc., Kankakee, Ill., USA). Lysozyme standard serum (Behringwerke AG, Marburg Lahn, BRD) was used as control for the lysozyme assay method.

Lysozyme assay

A modified agar diffusion method (15) was used. Suspension of killed *Micrococcus lysodeikticus* (Difco Laboratories, Detroit, Mich., USA) was mixed with molten 1% agar (Special Agar Noble, Difco) in phosphate EDTA-buffer (7), pH 6.2, at 56°C, making up a final concentration of 50 mg dried *M. lysodeikticus* per 100 ml 1% agar. Each of plastic dishes, measuring 85 mm in diameter, were filled with 13 ml of this mixture while horizontally levelled. Eighteen wells, 4 mm in diameter and 15 mm apart, were cut in the solid agar in a fixed pattern. In each well was applied 25 μl of the standard solutions or undiluted specimens of serum, milk or fecal extract, using Oxford Sampler with disposable plastic tips (Oxford Laboratories, Calif., USA). After incubation at 20°C for 7 hours the diameters of the lysis zones were measured directly with a ruler against light. The diameters were plotted on a standard curve made with thirteen different dilutions of albumen lysozyme (Eisai) in PBS; their concentrations ranged from 1.2 to 5 000 μg/ml. A standard curve was made for each set up of tests, and at least 2 standards were applied on each of the other dishes.

As control for the activity of the enzyme preparation, standard curves were made with egg-white lysozyme from other manufacturers (Sigma Chemical Co., St. Louis, Mo., USA, Calbiochem, Los Angeles, Calif., USA and Koch-Light Laboratories Ltd., Colnbrook, England).

Other methods

The pH of feces was measured by mixing fresh feces with an equal volume of distilled water. The solids were allowed to settle for 20 minutes after which an indicator paper (Lyphan, Dr. Gerhard Kloz, West-Berlin, Germany) was dipped into the liquid. The pH of defatted milk was measured directly with this indicator paper. The values obtained differed at most 0.5 pH unit from control measurements with a Metrohm pH-meter (Metrohm AG, Herisau, Switzerland).

Double diffusion in 1% agar was carried out with sera, milk, fecal extracts and standards of albumen lysozyme, against antisera to both human and albumen lysozyme.

Acta Pædiatr Scand 63 588–594 1974

LYSOZYMES IN FECES FROM INFANTS AND CHILDREN

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ABSTRACT Haneberg B. and Finne, P (Department of Pediatrics and the Broegelmann Research Laboratory for Microbiology The University of Bergen, School of Medicine Bergen Norway) Lysozymes in feces from infants and children Acta Paediatr Scand 63: 588 1974.—An agar diffusion technique seemed useful for the assay of lysozyme activity in serum milk, and extracts of freeze-dried feces. Normal levels of this activity were established before the method was applied to extracts of feces from premature infants receiving either human milk, with lysozyme activity of its own or cow s milk with or without added albumen lysozyme Results of these investigations, in addition to immunologic studies, using antisera to human and albumen lysozymes, indicated that human milk lysozyme and albumen lysozyme in cow's milk formula may pass through the infants intestinal tract Albumen lysozyme however will most often be inactivated before being excreted with feces. Low fecal pH was found in those receiving albumen lysozyme as well as in those receiving human lysozyme This may reflect the intestinal activity of the lysozymes. Low intestinal pH, brought about by lactulose in the cow's milk feed, did not in itself lead to increased lysozyme activity At present no advantage of adding albumen lysozyme to cow's milk formula has been substantiated

KEY WORDS: Lysozyme feces, infants, children

Lysozymes or muramidases represent a group of enzymes widely distributed in nature (5) Human lysozyme is found in cells serum and secretions (5) including milk (2) and sometimes in feces (1 6) The enzyme is more constantly found in feces from breast fed infants than in feces from infants fed cow s milk (9)

Lysozymes will lyse bacteria through their effect on the cell wall (5) Their action on *Micrococcus lysodeikticus* is most readily demonstrated and is used for assay of the lytic activity (7) *In vitro* pathogenic bacteria will be lysed by the lysozymes only after exposure to heat acid alkali or various chemicals (3 4 14 16) by which the substrate is probably un masked Antibodies together with comple ment also seem to render the cell wall accessible to lysozyme (5) Thus lysozymes may play an active role in the defence against bacterial infections

Lysozymes from various animal species differ as revealed by immunological methods although they all act on *M lysodeikticus* (5) Egg white is a rich source of lysozyme and is used for commercial preparation of the enzyme Human milk contains much more lysozyme than does cow s milk (2) Enrichment with albumen[1] lysozyme would therefore render cow s milk more similar to human milk with regard to lysozyme activity Cow s milk formulas for infant feeding with small amounts of lysozyme added are on the market in some countries Clinical benefits of such formulas are reported (12)

[1] Hen egg white

Table 1. *Lysozyme activity in sera and extracts of meconium and feces from healthy infants and children as well as in milk from healthy women measured by agar diffusion method using albumen lysozyme as standard*

None of the infants received human milk

Material	Age of individuals	No. of individuals: Total	No. of individuals: Lysozyme measureable	Lysozyme µg/ml: Mean	Lysozyme µg/ml: (Range)	Lysozyme µg/ml: S.D.
Serum	0 (cord blood) –13 years	95	95	152	(6–400)	91
Meconium	1–3 days	10	0			
Feces	1–4 months	17	10	5	(0–13)	
Feces	4–36 month	18	10	1.9	(0–8)	
Feces	3–13 years	15	3	0.7	(0–6)	
	Time after parturition	No. of samples				
Human milk	3 days to 6 months	73	73	536	(84–1000)	543

Lysozyme measurable ≥1 µg/ml.
Not detectable 0.
One standard deviation, S.D.

Lysozymes in milk

The stability of albumen lysozyme in the cow's milk preparation was ensured: the activity was not reduced with storage in a dry state at room temperature for up to 16 months. The temperature of the preheated water used for preparation of the liquid formula was not critical. Less than 10% reduction in lysozyme-activity was found when initial water temperature was 90°C and no loss in activity could be demonstrated with water temperatures at or lower than 70°C. Storage of the liquid formula for 4 days at 4°C reduced the activity 50%.

In vitro fecal extracts pH 5.5–7 seemed to influence the activity of both human and albumen lysozyme. Firstly the activity in the fecal extract–milk mixture was greater than expected, i.e. at a higher level than in the milk–PBS mixture (Fig. 3). Secondly at 37°C the activity was slowly reduced while in the control mixture with PBS the activity seemed even to increase. The various fecal extracts differed greatly with regard to their effect on the lysozymes. This effect was not clearly dependent on pH, as extracts with pH 5.5 was fully able to destroy the lysozymes while some extracts with pH 7 had no such effect. Trypsin had this same inhibitory effect on the lysozymes as was observed with some fecal extracts. No effect of pepsin was observed and none of these fecal extracts or the trypsin solution lysed the *M. lysodeikticus* in the agar. As is exemplified in Fig. 3 no difference between human milk lysozyme and albumen lysozyme in

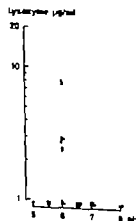


Fig. 2. Lysozyme activity in extracts of feces and pH in feces from healthy infants and children.

Table 1 *Lysozyme activity in sera and extracts of meconium and feces from healthy infants and children as well as in milk from healthy women measured by agar diffusion method using albumen lysozyme as standard*

None of the infants received human milk.

Material	Age of individuals	No of individuals: Total	No of individuals: Lysozyme measurable	Lysozyme µg/ml: Mean	Lysozyme µg/ml: (Range)	Lysozyme µg/ml: S D
Serum	0 (cord blood) –13 years	95	95	157	(62–400)	31
Meconium	1 3 days	10	0			
Feces	1–12 months	17	10	2 5	(0–13)	
Feces	1 36 months	18	10	1 9	(0– 8)	
Feces	3–13 years	15	3	0.7	(0– 6)	
	Time after parturition	No of samples				
Human milk	3 days to 6 months	73	73	556	(84–4000)	543

Lysozyme measurable ≥1 µg/ml.
Not detectable: 0
One standard deviation, S D

Lysozymes in milk

The stability of albumen lysozyme in the cow's milk preparation was ensured the activity was not reduced with storage in a dry state, at room temperature for up to 16 months The temperature of the preheated water used for preparation of the liquid formula, was not critical Less than 10% reduction in lysozyme activity was found when initial water temperature was 90°C and no loss in activity could be demonstrated with water temperatures at or lower than 70°C Storage of the liquid formula for 4 days at 4°C reduced the activity 50%

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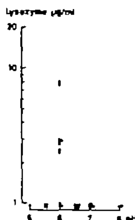


Fig 2 Lysozyme activity in extracts of feces and pH in feces from healthy infants and children

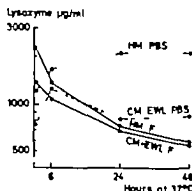


Fig 3 Lysozyme activity in human milk (*HM*) and in cow s milk formula (*CM*) with added albumen lysozyme (*EWL*) mixed with equal volumes of fecal extracts (*F*) or phosphate-buffered saline (*PBS*) pH 7 2 before and after incubation at 37°C for various lengths of time The whole lines are drawn between the mean of the values obtained with six fecal extracts.

cow s milk formula could be demonstrated by these experiments

Lysozymes in feces

In extracts of feces from infants fed human milk was found much greater lysozyme activity than in feces from infants on ordinary cow s milk formula (Table 2) Also significantly higher levels of lysozyme were demonstrated in feces from the individuals on lysozyme-enriched cow s milk compared to those on ordinary formula A wide range of activity was found in feces from the ones receiving either human milk or formula with albumen lysozyme However in feces from infants on human milk lysozyme was always measurable whereas in feces from infants receiving cow s milk with added albumen lysozyme measurable activity was not found more often than in the control group Double diffusion in agar of fecal extracts with high levels of lysozyme gave a precipitation line against antiserum to either human or albumen lysozyme As is summarized in Table 3 the use of these antisera made it possible to *distinguish between the two species-specific lysozymes* at least when the activity exceeded a certain level

The pH of feces was distinctly lower in the individuals fed human milk than in those on ordinary formula (Table 2) Addition of albumen lysozyme also seemed to lower fecal pH although the pH of feces from infants receiving albumen lysozyme was higher ($p<0.001$) than that of feces from infants fed human milk Lactulose added to the cow s milk formula lowered the fecal pH almost to the level found in infants receiving human milk (Table 4). However the lactulose did not influence the lysozyme activity of feces

The lysozyme activity in sera from all the

Table 2 *Lysozyme activity in extracts of feces and pH in feces from premature infants fed either human milk ordinary cow s milk formula or formula with added albumen lysozyme (400 µg/*ml)

The lysozyme values were based on standards of albumen lysozyme The calculated significance probability is given

Feeding	No of samples Total	Lysozyme measurable	Lysozyme µg/ml Mean	(Range)		pH Mean	(Range)	
Human milk	58	58	757	(2–3 500)	$p<0.001$	5 0	(4 –5.5)	$p<0.001$
Cow s milk formula	102	24	1 8	(0– 45)	$p<0.005$	6 4	(5.5–8)	$p<0.02$
Cow s milk formula with lysozyme	84	22	23 6	(0– 600)		6 1	(5–8)	

Lysozyme measurable≥1.2 µg/ml.
Not detectable 0.

Table 3. *Precipitation in agar (+) by double diffusion of standards of albumen lysozyme, albumen lysozyme in cow's milk formula, human milk and fecal extracts against antisera to either human or albumen lysozyme*

The lysozyme activities necessary to give visible precipitates, are given

Material	Lysozyme (μg/ml)	Antiserum to Human lysozyme	Albumen lysozyme
Albumen lysozyme in PBS or cow's milk formula	> 20	−	+
Human milk	> 30	+	−
Extracts of feces from infants fed			
(A) cow's milk formula		−	−
(B) Cow's milk formula with albumen lysozyme	> 200	−	+
(C) Human milk	> 400	+	−

premature individuals fell within the normal range for this method. No significant difference was found between the serum levels for the three feeding groups.

DISCUSSION

The use of an agar plate method seemed practical for the assay of lysozymes in different biological materials. The wide range measured without having to dilute or concentrate the liquid samples made the technique very simple. The precision was comparable to that of other methods (13), although an immunochemical determination ranged far better (11). As is also reported by some (15), the activity of human lysozyme measured in this way exceeded the values obtained with other methods (5). Therefore, the levels of lysozyme activity found in our series had to be compared with those of healthy individuals using the same method.

The lysozyme activity measured by a turbidimetric method correlated well with the concentrations obtained by an immunochemical method (11). However, our findings of increased levels of lysozymes in milk when mixed with fecal extracts indicate that *fecal components may activate the lysozymes or act on M. lysodeikticus to render these cells more prone to lysis.* The tendency to increased activity was also evident with milk incubated at 37°C, suggesting changes of the milk or lysozymes. Thus our method, going for the activity, may not reflect the true lysozyme concentrations. Since human and albumen lysozymes seem to behave in the same way when exposed to various environmental factors *in vitro* simulating conditions in the gut, the testing for their activity in feces may still be of value. Even more, the increased levels of lysozyme activity, and the inapparent or slow inactivation thereof when exposed to body temperature and fecal components, may possibly give a just picture of the activity *in vivo*.

Human milk lysozyme seemed to resist the conditions of the gastrointestinal tract

Table 4. *Lysozyme and pH in feces from 6 infants first fed ordinary cow's milk formula, thereafter formula with added lactulose*

The calculated significance probability is given

Formula	No. of samples	Lysozyme μg/ml Mean	(Range)		pH Mean	(Range)	
Without lactulose	12	0.8	(0–5)	$0.2 < p < 0.3$	7.0	(6.5–8)	$p < 0.001$
With lactulose	26	3.1	(0–8)		5.3	(4–7)	

Not detectable: 0

since measurable activity and mostly high levels were found in all extracts of feces from premature infants receiving that milk With albumen lysozyme it was different in only about one-quarter of the fecal samples was lysozyme activity measurable even though this enzyme was added to the formula in amounts making up an activity almost equal to the mean level found in breast milk In some however high levels were found along with the demonstration of albumen lysozyme by immunological methods This indicates that albumen lysozyme in cow s milk may pass through the infant gut Also the slightly lowered pH of feces from infants receiving lysozyme-enriched cow s milk suggests that albumen lysozyme may exert some effect in the gut before in activated or excreted Therefore the inactivation of albumen lysozyme in the gut of most individuals may be explained by prolonged exposure to contents of the gastrointestinal tract or by the effect of proteolytic enzymes which may be more potent at a higher pH

As expected additional lactulose by mouth lowered the pH of feces The lack of concomitant increase in fecal lysozyme activity indicated that low intestinal pH in itself will not bring about greater lysozyme activity This is further evidence for the assumption that most of the lysozyme in feces of breast fed infants is actually derived from human milk Thus lysozyme in human milk seems to be better preserved in the infant gut than is albumen lysozyme in cow s milk It remains to be seen however whether albumen lysozyme may influence the bacterial flora of the infants s intestinal tract The present study does not substantiate any great advantage with the addition of albumen lysozyme to commercial cow s milk formula

ACKNOWLEDGEMENTS

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ASPECTS ON REWARMING NEWBORN INFANTS WITH SEVERE ACCIDENTAL HYPOTHERMIA

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ABSTRACT Tafari, N and Gentz, J. (Department of Paediatrics, Haile Selassie I University Addis Ababa and the Department of Paediatrics, Karolinska Institutet, St Göran's Hospital for Children, Stockholm, Sweden) Aspects on rewarming infants with severe accidental hypothermia. Acta Paediatr Scand 63: 595 1974.—Thirty low birth weight infants with severe hypothermia corresponding to rectal temperature of <32°C (range 26.0°C–32.0°C) were studied. Fourteen infants were rewarmed according to a slow and 16 infants according to a fast procedure. The alleged disadvantages of fast warming over slow warming were not demonstrated. A significant fall in mortality was achieved in both groups following the intravenous administration of 0 15 M saline in a volume of 20 ml per kg bodyweight early in the rewarming procedure from 9 out of 12 dead to 3 out of 18. The reason for this effect of saline infusion is not clear but extrapolating from induced hypothermia restoration of plasma volume is a possible explanation. Factors such as birthweight, degree and duration of hypothermia were also important in influencing survival. Although there was no difference between the two methods in the outcome in this material, analysis of temperatures recorded during rewarming (rectal, skin and environmental air temperature) showed that the rapidly warmed infants increased their body temperature as a result of net transfer of heat from the ambient air In contrast, among the slowly warmed infants there was no demonstrable transfer of heat from the ambient air It therefore appears that the rapid warming of infants with severe hypothermia may have the advantage of minimizing additional energy expenditure for heat production

KEY WORDS: Accidental hypothermia, newborn infants, rewarming

Cold stress is now recognized as an important cause of death in the newborn infant (4 5 9 14) Profound hypothermia developing into the well known entity of neonatal cold injury (11) is the final result of cold stress where the capacity of heat production to maintain normal body temperature has been exceeded by heat loss To some extent this may occur at birth and during transportation even when care is taken to minimize heat loss (6)

The fully developed entity of neonatal cold injury has mostly been described from European countries and recently from North America (1 3 11 13) Although the physical conditions for heat loss during the newborn period exists in tropical climates severe hypothermia has not been recognized as a major problem in Africa. Neonatal cold injury was recently reported to be prevalent among low birth weight newborn infants in Ethiopia and the clinical manifestation as well as the outcome of therapy were similar to the reports from the United Kingdom (16)

The recommended method of raising body temperature of infants with profound hypothermia is gradual rewarming over the first 24 to 72 hours and fast rewarming over a few

since measurable activity and mostly high levels were found in all extracts of feces from premature infants receiving that milk With albumen lysozyme it was different In only about one-quarter of the fecal samples was lysozyme activity measurable even though this enzyme was added to the formula in amounts making up an activity almost equal to the mean level found in breast milk In some however high levels were found along with the demonstration of albumen lysozyme by immunological methods This indicates that albumen lysozyme in cow s milk may pass through the infant gut Also the slightly lowered pH of feces from infants receiving lysozyme-enriched cow s milk suggests that albumen lysozyme may exert some effect in the gut before inactivated or excreted Therefore the inactivation of albumen lysozyme in the gut of most individuals may be explained by prolonged exposure to contents of the gastrointestinal tract or by the effect of proteolytic enzymes which may be more potent at a higher pH

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Table 1 *Clinical data on 30 infants warmed by the two methods*

Figures within parentheses give range values

	Slow	Fast
No	14	16
Male	7	12
Female	7	4
Gestational age (wks)*	34 0 ± 2.7 (31–38)	34 8 ± 2.6 (31–38)
Birth weight (kg)*	1 47± 0.44 (0 81–2.29)	1.58± 0.47 (0 92–..46)
Length (cm)*	41 77± 3.37 (41–48)	42.50± 3 18 (41–47)
Age at admission (hr)*	29.86±25.86 (9–96)	26.75±29.50 (7–166)
Temperature at admission (°C)*	29 71± 2.26 (26–32)	30.41± 1.25 (?7–32)
Neonatal cold injury syndrome	5	4
Associated illnesses		
Asphyxia	3	7
IRDS	2	4
Anemia (venous Hb<13 0 g/100 ml)	–	2
Duration of warming (hr)*	19.00±11 76***	2 70±1.09***
Rate of rise of body temperature (°C/kg hr)*		
Skin	0.36± 0.32***	1 41± 0.51
Rectal	0.38± 0.31***	1.22± 0.37***
Terminal events at death		
Tonic seizures	2	
Apnea		1
Pulmonary haemorrhage		3
Outcome		
Lived	8	10
Died	6	6
% mortality	42.9	37.5

Values are given as means ±S.D

Significant differences between means ***$p<0.001$

rapidly rewarmed infants died corresponding to 42.9 and 37.5% mortality respectively The terminal events (Table 1) were similar in both groups

The mean ±S E M rectal and environmental temperatures during rewarming are depicted in Fig. 1 The mean gradients ±S E.M for skin temperature to environmental temperature as determined from incubator temperature (ΔT°C S-E) and for rectal temperature to skin temperature (ΔT°C R... have been calculated in the slow and th... rewarming groups As seen in Fig 1 B mean skin temperature in the slowly warmed group remained higher than th... environmental temperature throughout the re

Table 2. *Admission laboratory data on infants warmed by the two methods*

Values are given as mean ±S D The differences in the means between the two groups were not significant

Method of warming	Hgb (g/100 ml)	HCT (%)	Na (mEq/l)	K (mEq/l)	pH*	HCO- (mEq/l)	BUN (mg/100 ml)
Slow	18.71 ±3 1 (n=13)	58.. ±11 1 (n=13)	142.0 ±8 1 (n=11)	4.58 ±1.07 (n=9)	7.24 ±0.16 (n=12)	15 1 ±4 1 (n=12)	20.4 ±12.1 (n=11)
Fast	17 38 ±4.33 (n=13)	54.6 ±12.3 (n=13)	137 4 ±7.2 (n=11)	5.52 ±1.21 (n=10)	7 29 ±0 11 (n=10)	15 7 ±3.2 (n=10)	19 1 ±12.7 (n=13)

Values for pH were corrected for temperature using Rosenthal' factor

hours was widely condemned for reasons not altogether clear (1 3 11 13) Complica tions such as convulsions hyperthermia and lethal metabolic effects were ascribed to rapid warming Since slow rewarming over several days is difficult to control we em barked on a study to test the relative merit of fast and slow rewarming methods

MATERIAL AND METHODS

A total of 30 infants from Addis Ababa were studied. Most of the infants were born during the cold season when the temperature in Addis Ababa situated at a high altitude may drop to 5°C during the night This and poor housing was the likely explanation for the hypothermia Low birth weight associated with feeding dif ficulties was the most common reason for the parents to bring their infants to the hospital Hypothermia was regarded as severe when rectal temperature taken at least 7 cm from the anal orifice was 32.0°C or lower (range 26 0° to 32 0°C) The infants were assigned to either the fast or the slow warming group consecutively without any selection as to birthweight age or tempera ture at admission The clinical data on these infants are given in Table 1

Procedure All infants were placed in an intensive care incubator (AGA MK 41 AGA Inc Lidingo Sweden) The temperature in this incubator is maintained by heating the circulating air controlled either manually or by servocontrol via a thermistor attached to the infant s abdominal wall The thermistor was generally placed in the midline ? cm above the umbilicus. The environmental emperature was controlled within 0.5°C

T mperature recording All temperatures in the infants recorded by means of electrical thermometers ele Thermometer Model 43 Yellow Springs In ent Co Yellow Springs Ohio 45387 USA with uracy of 0 ?°C) or the built-in electric thermo- of the AGA incubator (accuracy ±0 2°C) En iro nental temperature was recorded from a Mercury thermometer in the incubator with an accuracy of ±0.5°C Rectal temperature was measured at least 7 cm from the anal orifice while skin temperature was regis tered in the midline 2 cm above the umbilicus. All tem peratures were measured at 15 min intervals for the first hour and at 30 min intervals up to 6 hours Then the temperatures were recorded at hourly intervals until 24 hours and thereafter four hourly until normothermia was achieved.

Humidity Relative humidity in the incubator was maintained between 50 and 60% depending on the en- vironmental temperature

Slow warming This was carried out by setting en vironmental temperature manually at the rectal tem perature of the infant and subsequently increased in a stepwise fashion until the thermoneutral zone of the infant was reached Since the rectal temperature at admission varied the time taken to reach the thermo- neutral zone (15) of the individual infants also varied Once the thermoneutral zone was achieved the infant was kept in such an environment until the end of the trial period, i e when normothermia was reached

Fast warming This was carried out by means of servo- control The servo-controlled thermostat was set to achieve abdominal skin temperature of 36 0°C. This meant that the heating unit of the incubator was work ing, at times with full capacity resulting in environ- mental temperatures as high as 38 0°C However with the rise of the infant s body temperature, the in- cubator temperature approached what is known to be the thermoneutral zone for the infant

Laboratory investigations At admission an umbilical venous catheter was inserted. Blood samples (3 0 ml) were withdrawn and put in heparinized test tubes. An aliquot was taken for haemoglobin and haematocrit de- terminations. These tubes were immediately centrifuged and plasma analysed for sodium potassium and blood urea nitrogen (BUN) Blood for pH and standard bi- carbonate determinations was obtained in heparinized capillary tubes sealed with plastiline and kept on ice and analyzed within 10 minutes. Determinations were performed according to standard procedures. (Values for pH were corrected for temperature using Rosenthal's factor)

Supportive measures At admission all infants re- ceived infusions of 10% glucose in water 100 ml/kg/ day via the umbilical venous catheter Oral feeding was started when normothermia was achieved. Individualized therapeutic measures were given on indication Later in the study plasma expanders in the form of saline 0 15 M in a dose of ?0 ml/kg body weight was infused rapidly at the start of the rewarming procedure.

The rewarming procedure was considered successful if the infant survived for at least 7 days after a state of normothermia had been achieved

RESULTS

Slow versus fast rewarming Of the 30 in fants 16 were rewarmed according to fast and 14 to the slow rewarming procedure The pertinent clinical data are given in Table 1 The laboratory findings on admission are given in Table 2 Except for metabolic acido- sis and slightly elevated BUN the rest of the laboratory findings were within normal limits As can be seen from Table 1 there was no significant difference between the slow and fast rewarmed group except for the dura- tion of warming and the rate of rise of skin and rectal temperature (°C/kg×hr) which were significantly different ($p<0.001$) Six of the 14 slowly rewarmed and 6 of the 16

Table 1. *Clinical data on 30 infants warmed by the two methods*

Figures within parentheses give range values

	Slow	Fast
No.	14	16
Male	7	1
Female	7	4
Gestational age (wks)*	34.0 ± 2.7 (31–38)	34.8 ± 2.6 (31–38)
Birth weight (kg)*	1.47 ± 0.44 (0.81– 29)	1.58 ± 0.47 (0.97–2.46)
Length (cm)*	41.77 ± 3.37 (41–48)	42.50 ± 3.18 (41–47)
Age at admission (hr)*	29.86 ± 25.86 (9–96)	26.75 ± 29.50 (7–166)
Temperature at admission (°C)*	29.71 ± [illegible].26 ([illegible]6–3[illegible])	30.41 ± 1.25 (27–32)
Neonatal cold injury syndrome	5	4
Associated illnesses		
Asphyxia	3	7
IRDS	2	4
Anemia (venous Hb<13.0 g/100 ml)	–	
Duration of warming (hr)*	19.00 ± 11.76**	2.70 ± 1.09***
*Rate of rise of body temperature** (°C/kg hr)		
Skin	0.36 ± 0.32**	1.41 ± 0.51 **
Rectal	0.38 ± 0.31 **	1.22 ± 0.37**
Terminal events at death		
Tonic seizures	2	
Apnea	2	1
Pulmonary haemorrhage	2	3
Outcome		
Lived	8	10
Dead	6	6
% mortality	42.9	37.5

Values are given as means ± S.D

Significant differences between means ** = $p<0.001$

rapidly rewarmed infants died corresponding to 42.9 and 37.5% mortality respectively. The terminal events (Table 1) were similar in both groups.

The mean ± S.E.M. rectal and environmental temperatures during rewarming are depicted in Fig. 1. The mean gradients ± S.E.M. for skin temperature to environmental tem- perature as determined from incubator tem perature (ΔT°C S-E) and for rectal tem perature to skin temperature (ΔT°C R have been calculated in the slow and th rewarming groups. As seen in Fig. 1 B mean skin temperature in the slow warmed group remained higher than th en vironmental temperature throughout the re

Table 2. *Admission laboratory data on infants warmed by the two methods*

Values are given as mean ± S.D. The differences in the means between the two groups were not significant

Method of warming	Hgb (g/100 ml)	HCT (%)	Na (mEq/l)	K (mEq/l)	pH*	HCO- (mEq/l)	BUN (mg/100 ml)
Slow	18.71 ± 3.1 (n=13)	58 ± 11.1 (n=13)	142.0 ± 8.1 (n=11)	4.58 ± 1.07 (n=9)	7.[illegible]4 ± 0.16 (n=12)	15.1 ± 4.1 (n=12)	20.4 ± 1[illegible].1 (n=11)
Fast	17.38 ± 4.33 (n=13)	54.6 ± 12.3 (n=13)	137.4 ± 7.2 (n=11)	5.52 ± 1.21 (n=10)	7.29 ± 0.11 (n=10)	15.7 ± 3.2 (n=10)	19.1 ± 12.7 (n=13)

Values for pH were corrected for temperature using Rosenthal's factor

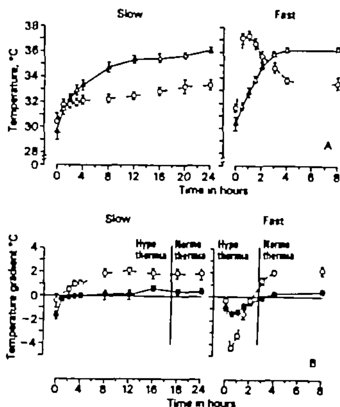


Fig. 1. (A) Mean ±S E M rectal (Δ—Δ) and environmental (O- -O) temperature during rewarming of the slowly and rapidly warmed infants. (For discussion see text.) (B) Mean ±S E M temperature gradients rectal–skin (●—●) and skin–environment (O- O) during rewarming of the slowly and rapidly warmed infants. The vertical line represents the mean time taken by the infants to reach normothermia.

warming period after the first hour In contrast the rapidly rewarmed group experienced a mean environmental temperature that was 4 46°C higher than the skin temperature after 30 min of warming Between the 2nd and 3rd hour of warming the skin temperature had exceeded the environmental temperature and remained so The thermoneutral zone was reached after a mean time of 7 0 and 3 4 hours in the slowly and rapidly warmed groups respectively

Saline treated versus the untreated The fast and the slow rewarmed groups were further divided into those who received and into those who did not receive saline at the beginning of the rewarming procedure The pertinent data are given in Table 3 When infants within each rewarming group were compared the only statistical difference found was the age at admission within the rapidly rewarmed group ($p<0.05$) Of the 12 infants that did not receive saline 9 died while 15 out of 18 saline treated infants survived

Survival versus death In Table 4 data on the infants who survived are compared with those who died As can be seen from this table the infants who survived were significantly heavier ($p<0.001$) younger on admission ($p<0.01$) and had a higher rectal temperature ($p<0.001$) However the rates of rise of body temperatures were not significantly different in the two groups

DISCUSSION

The present study does not confirm the advantage of slow rewarming over fast rewarming Since most of the infants were less than 30 hours of age and did not have the fully developed syndrome of neonatal cold injury we cannot disprove the advantage of slow rewarming in that condition The feared complications of fast warming namely convulsions (1 3 11 13) were seen no more often in the rapidly warmed infants than among the slowly warmed and in none of the infants who survived Similar findings were reported in a recent study of induced hypothermia in rabbits (17) Following a short period of hypothermia the survival rate was greater with rapid than with slow rewarming but following a 24-hour period of hypothermia this difference was not demonstrated

It has been found in the newborn infant that unless a positive gradient skin–environment of at least 2°C is maintained heat dissipation from the infant is hampered and core temperature will rise (12) It is interesting to note that in the slowly warmed group this gradient of 2°C is maintained although the infant is still in the hypothermic state Since these infants had a steady increase in rectal temperature (Fig. 1) with a gradient (skin–environment) found in normothermic infants dissipating heat there could

Table 3 *Comparative data on infants who received 0 15 M saline and those who did not*
Values are given as means ±S.D The only significant difference between the means of the different groups was for age at admission* within the fast-warmed group

Method of warming	No saline						Saline					
	No.	Weight (kg)	Age at adm (hr)	Rectal temp. (°C)	Result Lived	Result Died	No	Weight (kg)	Age at adm. (hr)	Rectal temp (°C)	Result Lived	Result Died
Slow	6	1.30 ±0 44	37 5 ±31 3	29 9 ±1.5		4	8	1.59 ±0 43	20.4 ±9 6	29 6 ±..4	6	
Fast	6	1 40 ±0.56	55 7* ±39 1	30 0 ±1 6	1	5	10	1.66 ±0 41	14 4* ±7 1	30 7 ±1 0	9	1

* $p<0.05$

not have been a transfer of heat from the environment to the infant Thus implies further that the heat must be internally produced It has been shown that when the environmental temperature is steadily increased as in the slowly warmed group oxygen consumption of the infant remains minimal and therefore heat production is minimal (8) If this is true the slowly rewarmed infants should not have increased their body temperatures The rise in rectal temperature of this group of infants therefore must at least partly be effected by increased heat production above basal levels

In contrast the rise in body temperature of the rapidly warmed infants was to a large extent the result of a transfer of heat from the environment to skin and skin to the core until normothermia was reached (Fig. 1) Once normothermia was achieved the environmental temperature stabilized within the "thermoneutral zone" and the gradient (skin–environment) became similar to that seen in the slowly warmed group indicating normal heat dissipation. From the above considerations it seems reasonable to assume that fast warming has the advantage over slow warming in minimizing unnecessary energy losses for heat production

In the early phase of the study the results were most discouraging, i e 9 out of 12 infants had died regardless of the method of warming The infants who died showed signs of circulatory disturbances and it is known from the findings in induced profound hypothermia in both man and the experimental animal that the systematic arterial pressure falls and plasma volume decreases Further cardiac output decreases and the total peripheral resistance increases (2 7 10)

Table 4 *Comparative data on infants that survived and died*
Values are given as mean ±S.D

Outcome	No	Birth weight (kg)	Age at admission (hr)	Temp. (TR) at admission (°C)	Rate of rise of temp. °C/kg hr Slow TS	Slow TR	Fast TS	Fast TR
Survived	18	1 73 ±0 41	15 83** ±8 69	30 83** ±0.77	0.26 ±0.19	0 28 ±0 22	1 42 ±0 48	1 19 ±0.37
					n=8		n=10	
Died	1	1 18* ±0 77	48 33 ±41 73	28 9** ±.. 04	0.49 ±0 41	0 57 ±0.38	1 40 ±0 6.	1.29 ±0 40
					n=6		n=6	

Degree of significance between means: ** <0 001 * <0 01

In animals that die during rewarming the above abnormalities persisted (7) With this knowledge in mind an attempt was made to restore the plasma volume using 0 15 M saline since plasma was not readily available Whether the dramatic effect on survival with this treatment was due only to restoration of the plasma volume or other factors were involved deserves further investigation

However the survival of the infants with profound hypothermia cannot be solely at tributed to the apparent beneficial effect of 0 15 M saline infusion Other factors such as birthweight degree and duration of hypo thermia were also important

ACKNOWLEDGEMENTS

We are grateful to AGA Inc Lidingö Sweden for providing their intensive care incubator MK41 for the purpose of this study This investigation was also supported by a research grant from the Haile Selassie I University

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INDIRECT MEASUREMENT OF SYSTOLIC BLOOD PRESSURE IN THE NEWBORN

A New Reliable Method

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ABSTRACT: Shaw A., Maxted, K. J. and Mcdonald, T H. (Department of Clinical Physics and Bio-Engineering Glasgow and Falkirk and District Royal Infirmary Falkirk, Scotland). Indirect measurement of systolic blood pressure in the newborn. A new reliable method. Acta Paediatr Scand, 63: 601 1974 —The accurate measurement of systolic blood pressure in the newborn by non-invasive means has, in the past, proved difficult. A new reliable method has been developed which employs a rigid hinged cuff to occlude the limb, and a thermistor circuit to detect the pulse. The performance of the device is compared with measurements obtained by direct arterial catheterization and its use in the routine clinical situation is evaluated. This device is significantly more convenient and reliable than present methods. It is small, self-contained and relatively inexpensive to produce.

KEY WORDS: Systolic blood pressure newborn, sphygmomanometer thermistor

The measurement of systolic blood pressure (S.B P) by non-invasive means in the new born has always proved troublesome A reliable indication of whether the limb is occluded or not is difficult to obtain partly because there is little or no patient coopera tion and partly because the pulse can be weak and therefore not easily detected (1 4) The relationship between actual blood pressure and cuff pressure can be variable dependent on a number of factors including the design of the cuff (3) and the delay in observing that blood has begun flowing in the partially occluded limb We carried out an appraisal of the various methods available and ex perienced difficulty in using all of them

Although the doppler shifted ultrasonic technique is a reliable and sensitive method (4) of detecting the pulse there are certain practical difficulties in its use It is awkward for one person to maintain the transducer over the artery and operate the sphygmo manometer bulb at the same time Alterna tively locating and fixing the transducer by a cuff or other means is time consuming and somewhat unreliable with infants who are not anaesthetized Adjusting the sound level whilst attempting to obtain a suitable posi tion for the transducer particularly when earphones are being used, is extremely in convenient The equipment required is expen sive and consists of quite a large number of component parts (i.e jel detector trans ducer earphones cuff manometer and bulb) which are not only time consuming to as semble but are also bulky and untidy in use

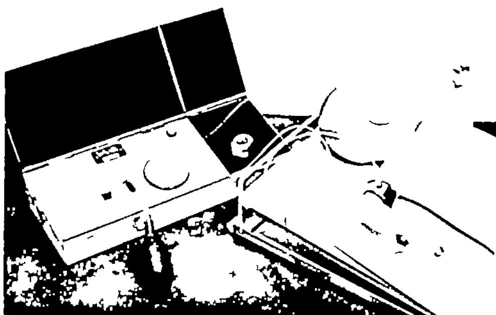


Fig 1 The thermistor sphygmomanometer

Methods employing strain gauges and photocells can be unpredictable mainly because of artifact effects caused by movement The time differential between recommencement of blood flow and indication can introduce substantial errors

We obtained reasonably good results with the xylol pulse indicator developed by Ashworth et al (1) The accuracy of this method was demonstrated by Bucci et al (2) by comparing results using a modified xylol pulse indicator with that of direct arterial cannulation They also showed that the instrument s performance was consistent when either the left or right upper limb was selected as the site of measurement However there are certain disadvantages in using this method in a routine clinical situation The excursion of the xylol beads is small and this caused some difficulty in deciding if blood was flowing in the limb It was awkward to move the beads into the capillary tube and maintain them there For the system to operate satisfactorily the capillary tube must be horizontal It is also fragile

We offer this new device as an alternative and practical method of obtaining accurate indication of blood pressure in the newborn and young child The prototype instrument has been in regular clinical use for several months

MATERIAL AND METHODS

The device consists of two basic parts The cuff and the pulse detector system As can be seen from Fig 1 the necessary sphygmomanometer equipment is also contained within the same unit There is storage space at the right hand end of the unit for the cuffs and tubing.

Two cuffs are used with our device The occluding cuff is placed in the normal position on the upper arm whilst the pulse indicator cuff is located around the wrist Both cuffs are similarly constructed and consist of rigid nylon outer rings hinged and secured by Velcro strips Soft rubber inserts are contained within the outer ring with a single connection for inflation Because the cuffs have rigid outer shells radial and longitudinal force components (generated during inflation of the occluding cuff) are not absorbed in deflecting that member

The indicator cuff is connected by a small-diameter plastic tube to a venturi tube containing a thermistor bead The thermistor is connected to a simple electronic circuit This circuit is comprised, as can be seen from Fig 2 of a field effect transistor constant current source driving a thermistor at about 1 mA This dissipates sufficient power in the thermistor to raise its temperature about 15°C above ambient Fluctuations in the air surrounding the thermistor in the venturi tube alter its dissipation changing its temperature and hence resistance It is insensitive to ambient temperature fluctuation The voltage across the thermistor is compared with that at a preset point on a potential

Fig. Details of the electronic circuit.

divider across the supply. The accuracy of the setting of this preset reference voltage is not critical as the amplifier is A.C. coupled to the thermistor. The A.C. coupling is designed as a differentiating element but also considerably aids setting up the circuit. It renders the circuit particularly sensitive to the rate of change of voltage typically produced by a pulse in the cuff but insensitive to a steady flow or only slow change in air velocity. The high frequency gain has been limited above about 20 Hz to improve stability.

The second, high voltage gain amplifier has also been A.C. coupled—considerably easing the design, and also allowing the response to be sharpened to give a sharp pulse output for the typical input signal. The final transistor ensures at least 0–5 V swing for a weak pulse to give a distinct display on the indicator lamp.

Care has been taken to keep battery consumption to a minimum and in operation the mercury cells employed should give at least 3 months reliable operation if the unit is not left on continuously. The indicator valve draws 150 mA from the 67.5 V battery and no more than 25 mA from the 1.5 V cell.

Inexpensive readily available integrated circuit amplifiers simplify the design to only a few components. This along with the small physical dimensions of the pneumatic system enables a compact portable instrument to be produced.

The device is used in the following way:

(i) Place cuffs around upper arm and wrist.

(ii) Switch on indicator and inflate cuffs to about 70–30 mmHg (on gauge).

(iii) Close valve (at top right hand section on panel) to wrist cuff when neon indicator bulb will commence flashing with characteristic pulse.

(iv) Continue to inflate until pulse indicator ceases to register.

(v) Release valve on bulb to allow pressure in the upper cuff to fall at a rate of about 2 mmHg per second.

(vi) When the indicator re-commences to pulsate read off pressure indicated on aneroid manometer. This is designated systolic blood pressure.

(vii) Open valve and remove both cuffs. Place these with the inflator bulb in the storage compartment.

CLINICAL TRIALS

We compared the performance of the instrument with that of intra-arterial S.B.P. The direct readings were obtained by Elema Schönander type E.M.T. 34 pressure transducer using an Argyle 5 F.G. 15 in type catheter inserted into the umbilical artery about 3 cm above the bifurcation of the abdominal aorta, with zero level set at the mid-axillary line. The frequency response of the catheter/transducer system was checked and found to be flat up to about 20 Hz. The infants included in this trial suffered from moderate to severe respiratory distress syndrome.

The recording of the arterial pressure waveform was displayed simultaneously with the pneumatic pressure in the occluding cuff, this reading being obtained by means of a pressure transducer (Elema-Schönander type E.M.T. 34). The output signal from the thermistor circuit to the indicator lamp on the instrument was recorded on the same paper chart.

We also assessed the indirect S.B.P. in both upper limbs of a random selection of 70 infants. The device was then used for routine blood pressure measurements for several months in a paediatric department.

RESULTS AND CONCLUSIONS

During the trial we experienced no difficulty in obtaining pulse indication and blood pressure measurements even in infants whose circulation was weak. Fig. 3 shows the relationship between the rhythmic pulsatile fluctuations in blood pressure by direct intra-arterial pressure recording and the display obtained from the indicator system with the occluding cuff deflated. The indicated and actual pulse frequencies are identical. This confirms that the indicator system is reproducing the pulsatile flow of blood in the limb at the site selected and is free from artifacts. When the occluding cuff is inflated to

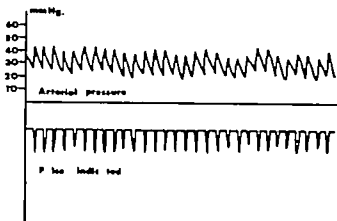


Fig 3 Pulse indicated with upper cuff deflated

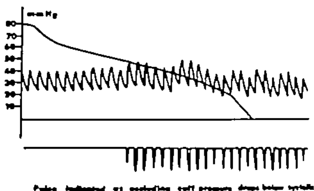


Fig. 4 Systolic blood pressure being measured.

a pneumatic pressure above systolic blood pressure it can be seen from Fig 4 that there is no cyclic output from the pulse indicator As the occluding cuff is slowly deflated blood flow recommences at a unique point on the pneumatic pressure trace The magnitude of the pneumatic pressure and that of actual blood pressure closely correspond demonstrating the accuracy of the instrument (Normally recommencement of blood flow would be indicated by the lamp flashing and the pneumatic pressure at which this occurs would be obtained by observation of the pressure reading on the aneroid manometer)

Paper chart records were obtained as previously described for a number of patients Accurate and reproducible results were obtained with the device The indicator system is sufficiently sensitive to detect short term transient fluctuations in blood pressure This is demonstrated in Fig 5 A line has been drawn through the actual blood pressure trace which corresponds to the pressure in the cuff Where actual blood pressure is greater than the occluding cuff pressure then an indicating signal is apparent and vice versa

From Table 1 it can be seen than the mean of the algebraic differences (*M*) was small indicating that there was no systematic difference between our device and direct S.B P or between indirect S P.B in the right arm and left arm It can also be seen that the standard deviation (S D) of differences was also small which indicates that good agreement was obtained between measurements simultaneously taken

The response of the indicator system is fast which is important if accurate results are to be achieved (For example assuming that the occluding cuff after inflation to its maximum pressure is allowed to decrease at a rate of 2 mmHg per second the response time of the instrument is 0 05 sec therefore the maximum error which can be attributed to this delay is 2 × 0 05 mmHg This we consider to be neglible) The overall precision of the instrument is mainly dependent on the accuracy in reading the aneroid manometer An accuracy of ± 1 mmHg can be obtained with the present manometer

The use of a thermistor to detect blood flow is not new (5 6) although our system is unique in certain respects The thermistor is sited in a venturi unlike the nozzle-jet system employed elsewhere Our design ensures good forward and reverse laminar flow characteristics of the air over the thermistor so that the electrical output from the indicator lamp is a true indication of the small volume changes within the cuff for the complete expansion/contraction cycle of the pulse in the member being observed This is essential if the arterial pulse is to be recognised and discriminated from artifacts produced by

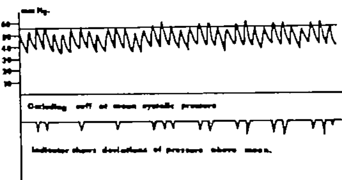


Fig. 5 Pulse indicated as peak systolic pressure varies.

limb movement and respiration The configuration of our pneumatic circuit is such that the capacitance of the reservoir and resistance between the reservoir and thermistor can be chosen to balance a wide range of lengths of tubing connected to the cuff

Our transducer is protected by placing it in the instrument box. The electronic circuit is specifically designed so that the output from the thermistor can be displayed by means of an inexpensive indicator (e g. a filament bulb).

Variations in the width of the occluding cuff do not affect the readings obtained Both cuffs are easy to use and can be applied with one hand only which is advantageous if the infant is in an incubator The cuffs can be positioned in about 2 to 3 seconds. This we consider important because the child is not really disturbed and the measurements can usually be carried out whilst he sleeps The cuffs can be cleaned speedily and effectively this being particularly relevant in an intensive care unit or in an operating theatre We are at present investigating the possibility of extending the size range of each cuff by utilizing a modified design based on the present one and incorporating both cuffs in a single unit.

The instrument can also be used in a simplified form (i.e. without inflator bulb aneroid manometer and valve) for use as a pulse indicator in adults for example during surgery

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Table 1 *Comparison between direct (aortic) SBP and indirect (arm) also between right and left arm (indirect method) by simultaneous measurements*

Method	No of cases	Body weight (kg, range)	Post-natal (age range)	Total no. of comparisons	Indirect SBP (mmHg, range)	Mean difference M (mmHg)	Standard deviation
Comparison of indirect to direct SBP	3	2 1–2.7	8–50 hours	30	36–74	−0 62	±1 31
Comparison of right–left arm SBP (indirect)	20	1.3–10.1	hours–200 days	100	40–128	−0.1	±2.15

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REVIEW ARTICLE

MATURATION OF CELLULAR AND HUMORAL IMMUNITY DURING HUMAN EMBRYONIC DEVELOPMENT

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INTRODUCTION

In this review the present-day information about the maturation of immunological capacities of T and B-lymphocytes during human embryonic development is summarized For this purpose the different sites of embryonic haematopoiesis the most likely stem cell candidate thymocytes T and B-lymphocytes are considered In addition the question of foetal sensitization by the mother is discussed Contrary to earlier beliefs both cellular and humoral immunity reactions develop early during human gestation.

The Changing Sites of Haematopoiesis

The first site of haematopoiesis in birds (65) and mammalian embryos (63) are the mesenchymal blood islands (66) of the yolk sac In man, haematopoiesis in the rudimentary yolk sac occurs between 3 and 6 weeks of gestation (Fig. 1) (110) Haematopoietic activity of the liver during human development is exclusively erythropoietic (104) Primitive erythroblasts are produced from about 4 weeks of gestation on (110)

In the human thymus lymphocytes are first seen at 9 weeks of gestation. From the 14th week on a cortical zone rich in mitotically active lymphocytes and lymphocyte precursor cells appears It reaches its maximum thickness at 20 weeks (72)

Evidence of a functioning bone marrow has been found at 11 to 12 weeks of gestation (85 115) From this time on the lymphoid compartment of the bone marrow forms about 25% of the total nucleated cells (115) Marrow haematopoiesis attains its maximum activity at 30 weeks of gestation (41) During the first 3 months of postnatal life bone marrow lymphoid cells show a steady rise and from the 4th week on lymphocytes form about 40–50% of all nucleated marrow cells (26 40) Bone marrow volumes of newborn infants amount to about 16–44 ml or 40% of skeletal volume This corresponds to 1 4% of the total body weight (37)

In the spleen lymphopoiesis is not evident at 11 gestational weeks (85) At 12 weeks scattered mononuclear cells are found in almost the entire pulp Between 12 and 15 weeks central arteriols appear which from 17 weeks on are accompanied by cuffs of small lymphocytes (2) Leukopoietic activity in the human spleen has its maximum at the beginning of the 5th month of gestation (68) but is much less pronounced than in laboratory animals (78)

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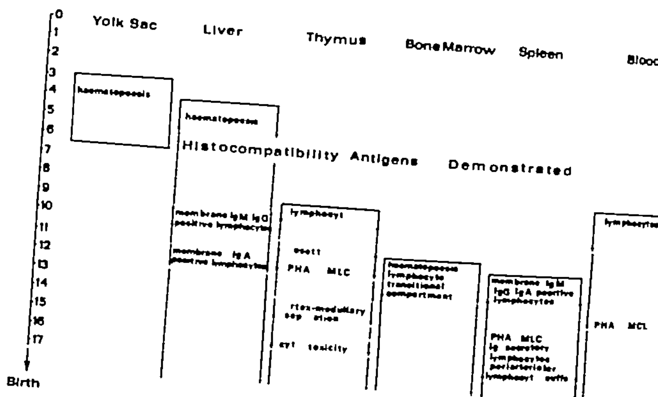


Fig 1 Times of appearance of haematopoiesis and of immunological potential in blood and various organs at different periods of gestation (*ordinate*) *PHA* = Lymphocytes responsive to phytohaemagglutinin *MLC* Lymphocytes responsive in mixed lymphocyte cultures

In the human appendix lymphocyte aggregates and primary follicles increase in the lamina propria after the 20th week of gestation till term especially in foetuses dying of infection The appendix behaves like a secondary rather than as a primary lymphatic organ (39)

Lymphocytes in Foetal and Newborn Blood

Lymphocytes are detectable in foetal blood at about 8–10 weeks of gestation (77 103) There is a rapid rise of lymphoid cell levels in the blood until the 25th week of gestation when a plateau of 2 5 to 3 thousand per mm³ is formed and maintained until birth Since active lymphocytopoiesis continues during the entire gestational period the plateau in the blood of lymphocyte levels must indicate a dynamic equilibrium between production and removal of lymphocytes from the blood (77 102 103)

At birth there are between 3 000 and 5 000 lymphocytes per mm³ of blood (18 103 112) After a transitory drop lymphocyte blood levels of 6 000 per mm³ are reached around the 10th day Premature newborn infants have somewhat lower blood levels depending on the length of their gestational periods (112)

By means of the leukophoretic effect of therapeutic exchange transfusions lymphocyte reserves of newborn infants have been determined as 25 4×10^7 cells per kg of body weight Exchange transfusions are followed by a lymphopenia reaching its lowest level after 17 to 40 hours after which blood lymphocytes rise again They continue to do so even beyond their normal levels after 96 hours

It is suggested that most of the mobilisable lymphocyte pool was released into the circulation during the exchange transfusion and that the subsequent increase in blood lymphocytes was due either to new cell formation or to the release of more slowly mobilisable cells (82 83)

Stem Cells

Evidence for circulating stem cells has been obtained from experiments with chick embryos (64 65 71) and with mammals (34 63 100) In addition irradiation protection experiments have shown that the protective effect of foetal and newborn blood lymphocytes is larger by a factor of one hundred to one thousand than that of adult leukocytes (4) Yoffey has pointed out clearly (114) that haemopoietic stem cells may also originate locally at least during embryonic development. For example during hepatic development, haemocytoblasts may have their origin from mesenchymal cells of hepatic trabeculae, as believed by Maximow (59 60) or in the case of human foetal liver they may possibly be derived from entodermal cells which have not yet differentiated into liver cells (101 104)

In the peripheral human blood there are normally 0 1% of mononuclear cells in spontaneous DNA synthesis (88) Newborn blood leukocytes have in vitro a higher spontaneous DNA synthesis rate (72, 111) than adult lymphocytes (53 81 84) Premature newborn infants have a much higher proportion of blood cells undergoing spontaneous DNA synthesis than premature infants studied at the time of their estimated birth dates or adults (81) By radioautography these cells have been identified in peripheral blood (21 22 111) and in bone marrow (116) to have a mean diameter of 10–14 μm, and a leptochromatic nucleus—in contrast to the small pachychromatic lymphocyte There is a high N/C ratio The cytoplasma may be pale or basophilic These cells have also been described in detail in guinea pig bone marrow (86) and have been designated transitional cells i e cells morphologically intermediate between lymphocytes and blast cells (114) During mid foetal life virtually all the circulating lymphocytes are of the transitional type (111) and in foetal bone marrow 15% of the lymphocyte population are transitional cells (101) They are very infrequent in adult blood (20 22) Transitional cells label immediately after ³H-thymidine injection except for the very early stages By contrast, labelled small lymphocytes are found only several hours after thymidine injection (69) Transitional cells—but not the small lymphocytes—must be an actively proliferating self-maintaining population

In conclusion, transitional cells and lymphocytes appear in appreciable numbers in the bone marrow and in the blood of foetuses when there is a peak demand for stem cells. There are no other cells present in sufficient numbers to serve as a stem cell

Although the morphological identity of the stem cell is still a matter of debate in the literature clinical observations yield additional information. De Vaal & Seynhaeve (107) described twins who had a complete absence of lymphocytes plasma cells and lymphatic tissue as well as a lack of granulocytes They have termed this syndrome Reticular Dysgenesia. Lamvik & Moe (50) have described an infant with a complete lack of an inflammatory response with granulocytopenia, thymic dysplasia and lymphopenia in the peripheral organs thrombocytopenia and haemolytic anaemia. Similar cases have been described by others (30 47 106) *In vitro* colony formation by stem cells from patients with aplastic anaemia is diminished (48) These clinical cases can be explained best by the assumption of a defect of haemato- lympho- and thrombocytopoietic stem cells

Thymocytes and T Lymphocytes

Haematopoietic stem cells migrate via the blood stream to the thymus (100) where they acquire surface alloantigens (70) The thymic cortex has a high proliferative activity (45) with a high rate of spontaneous *in vitro* DNA synthesis (2 74) During migration to the medulla (45) the cells undergo a process of maturation They become resist

ant to corticosteroids and acquire immuno competence (14) Salmonella flagellin bind ing thymic cells have been found in human foetuses of 20–22 weeks of gestation (19)

Cells leaving the thymus undergo a re arrangement of surface alloantigens e g by loss of TL-antigens According to a dif ferent concept only TL negative cells leave the thymus while TL positive cells die *in situ* (91) At any rate it is the TL negative thymus lymphocytes that mount a graft vs host reaction provide efficient helper cells for humoral antibody production (14) and that migrate to the thymic dependent areas of the peripheral lymph nodes (70) and the spleen These cells can be sensitized *in vivo* and *in vitro* against foreign trans plantation antigens after which they develop cytotoxic potentials (6 7)

Foetal thymocytes acquire PHA (phyto haemagglutinin) responsiveness at about 12 (2 11 38) to 15 (72) weeks of gestation This coincides with the demarcation of cor tex and medulla in thymic development (43) Between 15 and 20 weeks the response is variable (38) but sometimes very high (43) Two to four weeks after commencement of thymic PHA responsiveness splenic lym phocytes also respond to PHA This cor responds to the development of the thymic dependent cuffs surrounding central ar teriols of the spleen (2)

Blood lymphocytes acquire PHA respon siveness at 14 weeks of gestation (12) Blood lymphocytes of full term newborn infants (79 99 111) and of premature newborn in fants (79) react more vigorously than adult lymphocytes to stimulation by PHA and anti human lymphocyte globulin (ALG) (80) Other authors have seen no differences in response to PHA stimulation between new born and adult lymphocytes (53 57 58 75) or even a diminished response of newborn lymphocytes (3 38 62) These differences may be explained in part by differences in PHA dosages Ayoub & Kasakura (3) have found a factor in foetal plasma possibly a glycoprotein that suppresses the response of adult lymphocytes to PHA

Older premature infants i e premature infants studied at the time of their estimated birth dates show a reduced response to PHA and ALG stimulation as compared to the responses of full-term and premature newborn infants Their response is similar to that of adults (79 80)

Möller (61) has suggested that lymphocyte transformation may be a rather simple mem- brane activation phenomenon which occurs when a certain threshold number of surface sites is triggered Accordingly the differ ences in response to stimulation between in- fant and adult lymphocytes may be due to different sizes of PHA and ALG-sensitive blood lymphocyte populations of the dif ferent infant groups (79 80) Lymphocytes of infants with thymic dysplasia generally do not respond to PHA stimulation (32 33 36)

Mixed lymphocyte culture reactions (MLC) are closely linked with histocompati- bility differences between the reacting part ners (90) Seigler and Metzgar (93) have re ported the presence of histocompatibility antigens in a foetus of 6 weeks gestation. MLC reactions have been obtained with suspensions of foetal liver cells containing lymphocytes of 10 weeks of gestation (12) This is earlier than MLC reactions obtained in lymphatic organs and also earlier than PHA responsiveness Gatti and co-workers (27) have described an infant with the com bined defects of thymic aplasia and humoral antibody deficiency who showed a dissocia tion of PHA and MLC reactivity the former being absent while the latter was main- tained

Thymic lymphocytes reacting in MLC have been obtained at 12 (12 35) 13 (76) and 16 weeks (73) of gestation These cells are capable of mounting a graft vs host reac tion (76) Blood and splenic lymphocytes be come active in MLC reactions at 14–15 weeks (11) Cord blood lymphocytes are

more active in mixed lymphocyte cultures than adult lymphocytes (13 49)

Because of differences of only one HL A haplotype in father–child combinations reactions of cord blood lymphocytes against father's lymphocytes are weaker than against foreign lymphocytes (13) Cord blood lymphocytes react less vigorously to maternal than to paternal lymphocytes (13 52) This hyporeactivity is intensified by the addition to the cultures of maternal plasma, which appears to contain a factor inhibiting MLC as well as antigenic and PHA reactions of her newborn (52)

Foetal cells serve as stimulator cells as effectively as adult lymphocytes (13 73)

In the presence of PHA lymphocytes from foetuses of 16 weeks gestation (35) and from cord blood (10 99) are capable of cytotoxic lysis of xenogeneic cells Some rosette formation is found already at 11 weeks of gestation (99) It must be concluded from these data that cellular immune reactions develop early during gestation.

Foetal Sensitization

The question has been raised to what an extent the admixture of maternal cells to cord blood lymphocytes may play a role in studying cord blood leukocytes *in vitro* Sensitization of foetal lymphocytes by the mother against purified protein derivative (PPD) (1 23) and *E. coli* (9) has been reported Patients with congenital isolated IgA deficiency can synthesize *in utero* specific IgM or IgG antibodies against maternal IgA (108) On the other hand cells with a female karyotype among cord blood lymphocytes of *healthy* male newborns have not been found so far (56 67 94 105) As a hypothesis the mechanism of foetal sensitization may be by passage through the placenta of subcellular maternal informational material (23) or of antigen (28 55)

The opposite—passage of foetal lymphocytes into the maternal circulation—apparently does occur (17 92 109) In this way sensitization of the mother occurs with production of enhancing factors which interfere with the expression of maternal cellular reactivity against stimuli such as cells of the offspring

Plasma Cells and B Lymphocytes

Plasma cells and germinal centres (41) are not found normally during foetal life (8 77) or in full-term newborns (26 31 46) Plasma cells appear in the bone marrow (98) and intestinal mucosa (8) between 4 and 8 weeks postnatally Under pathological conditions such as congenital syphilis toxoplasmosis (95 96) or rubella (97) however plasma cell formation and an accelerated production of lymphoid nodules and reaction centres occurs

B-lymphocytes with membrane bound IgM and IgG are occasionally found in liver cell suspensions of only 9 $^1/_2$ weeks (51) and much more regularly in spleen cultures at 12 weeks of gestation (24) IgA positive cells are first detected at 11 $^1/_2$ weeks (51) By 14 $^1/_2$ weeks the percentage of cells in spleen and peripheral blood staining for IgM IgG and IgA was found to be within the normal range for full-term newborns healthy children and adults i.e IgM about 9% and IgG (mostly IgG_2) 2 9% (89) to 7 9% (24) Ruge and co-workers (89) have found 14.5% of lymphocytes of full-term infants to be IgD-positive

B-lymphocytes containing secreted cytoplasmic γ-globulin are found by 15 $^1/_2$ weeks of gestation they remain few in number until birth (51) Active immunoglobulin synthesis at a large scale in premature infants is related to birth and not to gestational age i.e it is a result of antigenic stimulation (42 87) Premature infants vaccinated with diphtheria toxoid at the time of their estimated birth dates are better antibody producers than full-term newborn infants On the other hand there is no significant difference in antibody responses be

ant to corticosteroids and acquire immunocompetence (14) Salmonella flagellin binding thymic cells have been found in human foetuses of 20–22 weeks of gestation (19)

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tween premature and full term infants immunized at birth (16) except for IgM production of infants with very low gestational ages (5)

Serum levels of IgG increase very slowly from 52 mg/100 ml of blood at 5 1/2 weeks of gestation to 180 mg/100 ml at 22 weeks The increase is most pronounced during the last weeks of gestation almost all IgG in foetal serum being of maternal origin (29) In full term infants serum IgG is 1100–1400 mg/100 ml of blood corresponding to the maternal blood level Lowest IgG levels are reached at 5 months of age with 400 mg/100 ml (42) Cord blood IgM is 10–14 mg/100 ml in healthy infants (15 113) rising to 90 mg/100 ml of blood by the end of the first year (42) This increase will however be considerably delayed in very premature infants (5) Serum IgA and IgD are not (25 89) or only rarely (15) found during foetal life IgA appears during the first weeks of postnatal life (42) Following oral immunization of newborns with life polio vaccine (44) or with bovine serum albumin (87) IgA production by the intestinal mucosa is stimulated

In accordance with the notion that antigen stimulated lymphocyte cultures detect secondary responses cord blood lymphocytes do not respond to primary antigenic stimulation (54) On the other hand immunization of newborn infants with typhoid vaccine results in transformation of blood lymphocytes of a similar magnitude as in lymphocytes from adults (53)

CONCLUSIONS

1 Transitional cells appear to be the most likely stem cell candidate after the establishment of definite haematopoiesis

2 The capacity of cellular and humoral immune reactions develops between 9 and 15 weeks of gestation but antigenic stimulation is necessary for humoral antibody production on a large scale

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tween premature and full term infants immunized at birth (16) except for IgM production of infants with very low gestational ages (5)

Serum levels of IgG increase very slowly from 52 mg/100 ml of blood at 5 1/2 weeks of gestation to 180 mg/100 ml at 22 weeks The increase is most pronounced during the last weeks of gestation almost all IgG in foetal serum being of maternal origin (29) In full term infants serum IgG is 1100–1400 mg/100 ml of blood corresponding to the maternal blood level Lowest IgG levels are reached at 5 months of age with 400 mg/100 ml (42) Cord blood IgM is 10–14 mg/100 ml in healthy infants (15 113) rising to 90 mg/100 ml of blood by the end of the first year (42) This increase will however be considerably delayed in very premature infants (5) Serum IgA and IgD are not (25 89) or only rarely (15) found during foetal life IgA appears during the first weeks of postnatal life (42) Following oral immunization of newborns with life polio vaccine (44) or with bovine serum albumin (87) IgA production by the intestinal mucosa is stimulated

In accordance with the notion that antigen stimulated lymphocyte cultures detect secondary responses cord blood lymphocytes do not respond to primary antigenic stimulation (54) On the other hand immunization of newborn infants with typhoid vaccine results in transformation of blood lymphocytes of a similar magnitude as in lymphocytes from adults (53)

CONCLUSIONS

1 Transitional cells appear to be the most likely stem cell candidate after the establishment of definite haematopoiesis

2 The capacity of cellular and humoral immune reactions develops between 9 and 15 weeks of gestation but antigenic stimulation is necessary for humoral antibody production on a large scale

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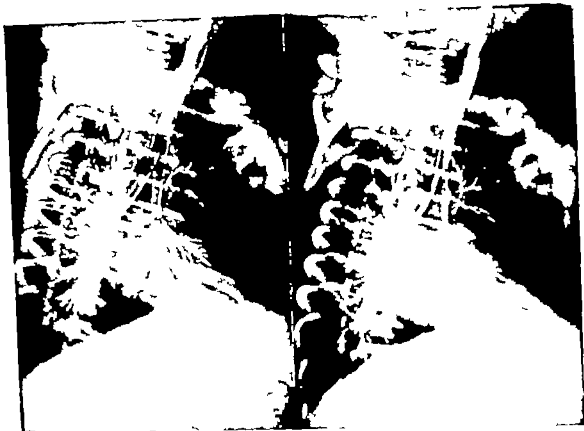


Fig. 1. Marked reduction of bronchial calibre during expiratory phase (*left*).

ted by endoscope could be explored without difficulty: the carina was displaced and the left main bronchus was found to collapse almost completely during expiration.

Bronchography: widening of bronchial branchings due to emphysema. For technical reasons, filling was limited to the large bronchi and showed no significant structural anomalies. Bronchial calibre was shown to reduce considerably during the expiratory phase.

Therefore the radiological picture strengthened the clinical suspicion of diffuse bronchomalacia (Fig. 1).

In accordance with the diagnosis of bronchomalacia, strongly suggested by both endoscopy and radiography, corticoid therapy was discontinued and treatment was reduced to general sedation and O_2 controlled administration.

Good results became soon evident: weight increased (7 kg at the age of 6 months), the respiratory conditions ceased to be critical, though a condition of mild respiratory distress persisted for a considerable period of time. However, the child could be kept out of the oxygen tent for increasingly long periods of time and sedation could be dispensed with. With passage of time, the child became interested in his environment and started to socialize. At the age of 7 months, he started to feed by himself and finally became self-sufficient at the age of 10 months, such as to be sent home. A mild respiratory acidosis, with 48–50 mmHg P_{CO_2} was always present.

During this period, the child developed several episodes of bronchopneumonia (caused by *Proteus vulgaris* and *Pseudomonas aeruginosa* shown in the fluid aspirated from the bronchi and in the pharyngeal swab) which were treated by Gentamycin (given both intramuscularly and by aerosol); later the infection was sustained by an *Alcaligenes fecalis*.

It is interesting to note that Pseudomonas colonies, as happens in mucoviscidosis, were particularly viscous, probably due to formation of a capsule. This may explain the particular density of the mucus.

DISCUSSION

There is little doubt that the severe chronic respiratory distress of our patient was due to an abnormal flaccidity of the bronchial wall as shown by bronchoscopy and bronchography. The increase of the transthoracic pressure during expiration nearly caused collapse of first and second generation bronchi.

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CASE REPORT

GENERALIZED FAMILIAL BRONCHOMALACIA

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ABSTRACT Agosti E. De Filippi G., Fior R. and Chiussi F (Paediatric Clinic University of Trieste Departments of Radiology and Otorhinolaryngology Istituto per l'Infanzia and Department of Paediatrics, Ospedale Civile Palmanova, Trieste Italy) Generalized familial bronchomalacia. Acta Paediatr Scand 63: 616 1974.—Generalized bronchomalacia was found in a 4-month-old infant with chronic respiratory distress since the first day of life The diagnosis was confirmed by endoscopy and radiography The patient differs in several respects from other cases reported. The disorder seems to be familial. The clinical symptoms were unusually severe and no evidence of bronchiectasis could be demonstrated.

KEY WORDS: Bronchomalacia

An abnormal weakness of a portion of the bronchial wall has been described both in cases of partial air trapping (localized emphysema) and in cases of generalized air-trapping (Williams & Campbell s syndrome) This report deals with a case of air trapping caused by generalized bronchomalacia. Some aspects of our case are similar to those described by Williams & Campbell (3) though some other less relevant features are different However a unique observation was the familial occurrence here represented

CASE REPORT

D M (clin rec 1538/70) is the fifth baby of healthy parents who are not known to be consanguineous although they have the same family name and both originate from the same small village Three out of four brothers of our patient died during the second month of life with severe dyspnoea arising soon after birth.

Our patient had severe breathing difficulty starting on the first day of life and had therefore been repeatedly referred to two different hospitals When he was sent to the Trieste Paediatric Clinic his age was 4 months and the body weight 3400 g

Blood count sweat test duodenal enzymes immunoglobulins and α-antitrypsin levels were found to be normal Only a few eosinophils were present in the peripheral blood and skin tests for allergens were negative

An unusually enlarged and radiolucent lung and an enlargement of the heart area were found at chest X ray examination. ECG showed a sinus rhythm with tachycardia as well as signs of right ventricular strain.

The child was first treated as a severe case of asthma and given antibiotics corticosteroids and bronchodilators but this treatment failed to improve his condition. Feeding had to be administered by gavage.

As the condition was a severe one and general health deteriorated it was decided to perform bronchoscopy and bronchography

Bronchoscopy no abnormality of the larynx a fibrous ring was found in the subglottic region, extending from 1 to 3 o clock causing a very limited stenosis The tube was passed over this obstacle with some difficulty and in the trachea a considerable amount of mucopus was found (microscopic examination showed only the presence of mucus cells and of some leucocytes, a Staphylococcus was found and antibiotic therapy was modified in accordance with the antibiogram) the orifice of the right main bronchus was quite normal and the right bronchial tree, although obstruc

CASE REPORT

COR TRIATRIATUM IN A 3 MONTH OLD INFANT

An operable form of pulmonary hypertension

FELIX WYLER, M. RUTISHAUSER and E. GRÄDEL

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ABSTRACT Wyler F Rutishauser M. and Grädel, E. (Children's Hospital and Department of Heart Surgery University of Basel, Switzerland). An operable form of pulmonary hypertension: Cor triatriatum in a 3-month-old infant. Acta Paediatr Scand, 63: 619 1974. —In a 3-month-old infant the clinical picture and the diagnostic criteria of cor triatriatum are presented. In addition to the pressure differences between PC wedge and left atrium the angiocardiogram is pertinent for detecting this malformation. This easily operable condition should be included in the differential diagnosis in every case of pulmonary hypertension from elevated pulmonary venous pressure. This is, to our knowledge the youngest patient reported with correct preoperative diagnosis and surgical cure.

KEY WORDS: Congenital heart disease, cor triatriatum, pulmonary hypertension

Cor triatriatum is a rare congenital heart anomaly where the left atrium is divided into two chambers by a fibromuscular membrane The pulmonary veins enter the proximal chamber and the distal part communicates with the mitral valve and the left atrial appendage. In the classical form the proximal chamber does not communicate with the right atrium an open foramen ovale however may be present between the distal left atrium and the right atrium. The small opening in the dividing membrane causes pulmonary venous congestion and consecutive pulmonary hypertension (3) Since it is an operable condition, recognition of this anomaly is of great importance We therefore report on diagnosis and treatment of this malformation in a 3-month-old infant.

CASE REPORT

The boy was admitted because of feeding difficulties and rapid respiration. He had tachypnoe of 80/min peripheral cyanosis, pallor and was irritable and sweating. The liver was 2 cm below the costal margin, the peripheral arterial pulses were normally palpable There was a marked right ventricular heave. At auscultation the first sound was normal, a faint systolic murmur was heard over the pulmonic area, the second sound was markedly accentuated and no splitting could be detected. A third sound was heard over the left lower sternal border The ECG showed atrial hypertrophy which was mainly right sided, and right ventricular hypertrophy with ST–T changes (Fig. 1). The chest X-ray revealed moderate right-sided cardiac enlargement with pulmonary venous congestion.

The clinical diagnosis was: pulmonary hypertension due to pulmonary venous congestion. The baby was put on digoxin and diuretics, and was catheterized one day later The hemodynamic data were as follows

The oxygen saturation of the right heart varied between 62 and 67% the values of LA and LV were normal (99%). The pressures were as follows. RA

Collapse leads to air trapping and severe expiratory distress simulating asthma the only effective management during the more severe stages consisted in deep sedation which abolished crying cough and respiratory effort and the expiratory pressure increase in the lung In this way we succeeded in keeping up feeding and growth for a few months progressively the size of the bronchi and—very likely—their strength increased and the child became gradually self sufficient

We think that our case differs from those described by Williams & Campbell and from other similar cases reported in the literature (2-3)

(*a*) The disease appears to have a familial character affecting 4 out of a total of 5 brothers Although we lack any evidence the parents might be consanguineous and it might be assumed that bronchial flaccidity is due to a rare recessive character

(*b*) The clinical pattern was more severe than the typical one of Willams and Campbell s syndrome three brothers died within the second month of life and our patient has been in constant danger during the whole of the first 6 months

(*c*) Bronchomalacia affected first and second generation bronchi whereas in the Williams & Campbell s syndrome only lesser bronchi are found to collapse

Surprisingly no clear-cut evidence of bronchiectasis (which is always present in the Williams & Campbell s syndrome) was found However slight irregularities of the bronchial wall suggest that such changes may arise in the future

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CASE REPORT

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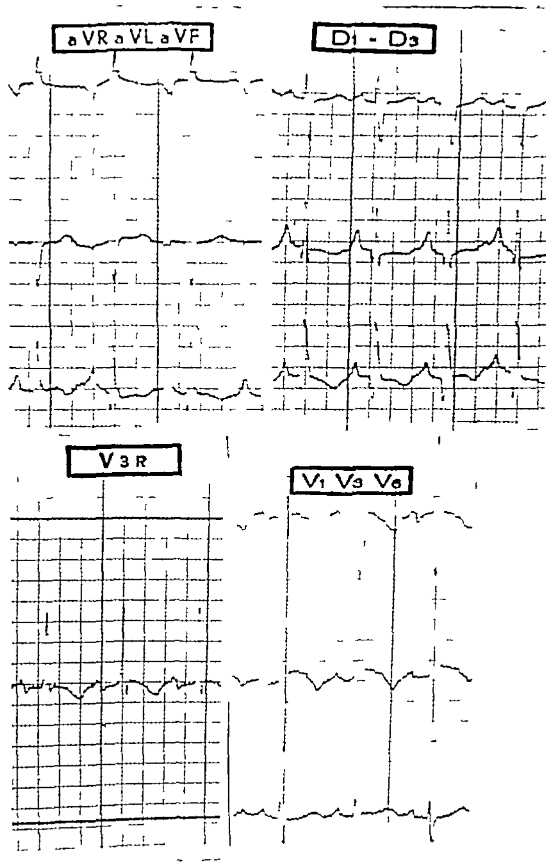


Fig 1 ECG on admission showing right atrial and right ventricular hypertrophy with strain



Fig. 2. Cine-frame with the injection of contrast material into the distal left atrium. Note the small size compared with Fig. 3.

= 6, *m* = 6–7 RV 60/6 PA mean *st.* 60/30, *m* 45 PA 60/35 l PA 60/37 PC wedge *a* = 19 *m* = 17 LA. *a* = 5 = 9 *m* = 7–8 LV 60/6 mm Hg.

Cineangiocardiography (injection into pulmonary artery) in the *p-a* view showed a very prolonged passage of the contrast material through the pulmonary circuit. The pulmonary veins entered a large left atrium. In contrast to the normally contracting left ventricle the systolo-diastolic movements of the left atrium were very poor. An injection into the part of the left atrium which was reached through the foramen ovale from the right atrium revealed an extremely small chamber (Fig. 2). This chamber consisted mainly of the left atrial appendage and showed immediate good emptying into the left ventricle. The size of the distal left atrial chamber was approximately 1/4 of the left atrial-size as seen from the pulmonary artery-injection (Fig. 3).

The pressure difference between pulmonary arterial wedge and the left atrium, and the cineangiographic evidence of a two-chambered left atrium were conclusive for the diagnosis cor triatriatum. 12 hours later surgery was performed, and the obstructing membrane was excised in normothermia and inflow occlusion. The opening was 4 mm in diameter. The postoperative course was uneventful, and the infant is doing well 7 months after the operation.

DISCUSSION

The difficulties encountered in handling this disease are not those of surgical treatment but of the correct preoperative diagnosis. Up till 1968 (1–6) there were only 66 cases of



Fig. 3. Visualization of the left atrium, proximal and distal part, from the injection into pulmonary artery.

cor triatriatum reported and of these only in 18 was a correct diagnosis made at operation or preoperatively Of these correctly diagnosed cases only 10 were detected in childhood and the youngest of these was 12 ½ months old In recent years the total number of correctly diagnosed cases reported in childhood increased to approximately 20 (2 4 5) our 3-month-old infant seems however to be the youngest patient where diagnosis and successful surgical treatment were accomplished Early recognition of the disease with prompt removal of the obstructive membrane are the prerequisites for reversibility of the high pulmonary vascular resistance and subsequent definite cure

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Acta Pædiatr Scand 63 623–626 1974

CASE REPORT

THE CAT EYE SYNDROME WITH UNUSUAL SKELETAL MALFORMATIONS

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ABSTRACT Balci, S., Halicioglu, C., Say B. and Taysi, K. (Department of Pediatrics and Division of Clinical Genetics, Hacettepe University Ankara, Turkey). The cat-eye syndrome with unusual skeletal malformations. Acta Paediatr Scand 63: 623, 1974.—A fifteen day old female infant with anal atresia, rectovaginal fistula, short left forearm and agenesis of the left thumb is presented. In addition, the patient had a small extra chromosome which was associated with D or G group chromosomes. The skeletal survey revealed aplasia of the first rib on the right as well as aplasia of the radial bone, first metacarp and thumb on the left. These radiological findings associated with the cat eye syndrome have not been reported previously

KEY WORDS: Cat-eye syndrome, polydactyly imperforate anus, vertebral anomalies syndrome radial agenesis, extra chromosome

The association of an extra small acrocentric chromosome with a spectrum of malformations was first described as a new entity by Schachenmann et al in 1965 (12) Since then sporadic as well as familial cases from various parts of the world have been reported (1 2, 5 7 8 12, 13 14). The characteristic associated findings of the syndrome are as follows. imperforate anus vertical coloboma of the iris, bilateral preauricular skin tags or fistulae congenital heart disease urinary tract malformations and mild to moderate mental retardation

The purpose of this communication is to report another such patient who had in addition serious skeletal malformations not described previously

CASE REPORT

A fifteen-day-old female infant was admitted with complaints of cyanosis, anal atresia and abnormalities of the left hand and arm The mother who was primipara, was [illegible] years old the father was 31 years old



Fig 1 X-ray of the arms. Note the absence of the radius, the first metacarp and the thumb on the left.

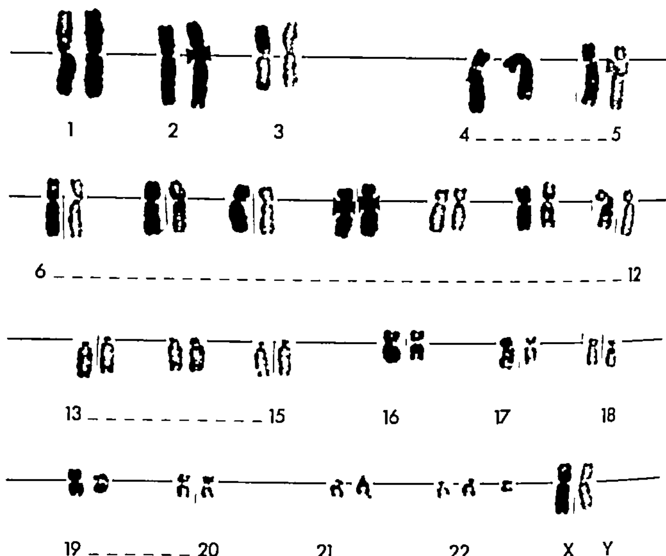


Fig 2 Karyotype of the patient

and had a normal male child from a previous marriage The mother had had a chest X-ray during the third month of pregnancy

Physical examination. The patient s weight was 2 500 g, height 47 cm and head circumference 35 cm. No abnormality of the eyes or ears could be detected. In addition to anal atresia and rectovaginal fistula the left forearm was shorter than the right and there was agenesis of the thumb on the left side Diastasis recti and mild umbilical hernia were also present

Laboratory findings Electrocardiogram showed right ventricular hypertrophy X-ray studies revealed a right aortic arch and possible tetralogy of Fallot. Other significant findings were aplasia of the first rib on the right as well as the radial bone and aplasia of the first metacarpal and thumb on the left (Fig. 1) The bone age corresponded to the eighth month of intrauterine life

Chromosomal analyses using peripheral leucocytes showed a small extra sub-acrocentric chromosome in 28 metaphase plates analysed (47,XX+mar?) (Fig. 2). An additional survey of 50 of the patient s metaphases revealed the following an extra chromosome smaller than the G group was observed in all but one of the metaphases in 33 of the metaphases this extra chromosome was associated with D or G group chromosomes (66% association) The mother s chromosomes were normal 46,XX in 30 metaphases The father was not available for chromosome analysis.

DISCUSSION

The patient had most of the cardinal findings of the cat-eye syndrome such as imperforate anus and congenital heart disease in association with a small extra chromosome in her

blood cells Although many patients with this syndrome also display coloboma of the iris and preauricular skin tags and fistulae these were not present in our patient. It should be remembered however that many cases lacking one or more of the cardinal findings have previously been reported (5) An interesting finding in our patient, not previously reported, was the presence of bony aplasia involving the forearm and thumb on the left. The first rib on the right was also absent Since association of certain skeletal anomalies including hypoplasia or an extra digit on the radial side and vertebral anomalies with imperforate anus has already been reported as a new syndrome (11) one is tempted to speculate a possible relation between these two conditions (10) It may be that some of the patients with the syndrome described by Say & Gerald represent cases with similar extra chromosomal material attached to another chromosome but the amount of chromatin involved is too small to be visualized with existing techniques It is also interesting in this respect that cases with findings commonly observed in the cat-eye syndrome but without the extra small chromosome have already been published (4 6) On the other hand it should be remembered that none of the typical cases with polydactyly/imperforate anus/vertebral anomalies syndrome so far reported showed iris defect or chromosomal abnormalities (9) while vertebral anomalies were found in only one possible and one definite case of cat-eye syndrome (6 13) and radial anomalies in none

The extra chromosome associated in most cases of the cat-eye syndrome has been variously reported as acrocentric (5) metacentric (1) or sub-metacentric (3) Furthermore satellites have been reported on the short arms (2) on the long arms (3) or not at all In the present case no distinct satellites were observed, but the high degree of association of the cat-eye chromosome with D or G group chromosomes suggests their existence. The only point of agreement about the cat-eye chromosome is that it is smaller than the G group chromosomes

Bühler et al (2) suggested on the basis of fluorescent staining properties that the extra chromosome might be derived from G_{22} Since some of the features involved are also commonly seen in D_1 trisomy it may be that the extra marker chromosome is a translocation product involving the distal part of the long arm of 22 and the short arm (including part of the centromere) of 13 Such an arrangement could explain in part the apparent polymorphism of the marker since the short arms of the D group chromosomes are known to exhibit polymorphism depending on techniques used. This could also explain the association phenomenon as well as the weak fluorescence of the cat-eye chromosome We hope new cytogenetic techniques may shed light on the nature of the chromosomal anomaly involved in this syndrome

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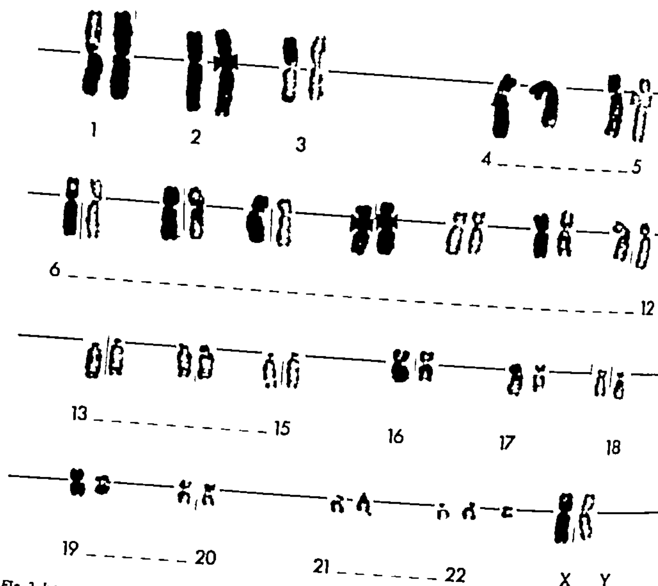


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CASE REPORT

PRESUMED DISSEMINATED BCG IN A BOY WITH CHRONIC GRANULOMATOUS DISEASE OF CHILDHOOD

PAULA VERRONEN

From the Department of Paediatrics (Head E. K Ahvenainen) the Central Hospital of Tampere Finland

ABSTRACT Verronen, P (Department of Paediatrics, Central Hospital Tampere Finland). Presumed disseminated BCG in a boy with chronic granulomatous disease of childhood. Acta Paediatr Scand 63: 627 1974.—A male child suffering from chronic granulomatous disease of childhood, confirmed by leucocyte bactericidal function test and a nitroblue tetrazolium test, was given a BCG inoculation in the neonatal period. He had draining lesions at the sites of vaccination and later miliary infiltration of the lungs, which responded favourably to antituberculous therapy Cultures for tb-bacilli were negative. An older sister is suspected to be a carrier of the trait responsible for chronic granulomatous disease. Her sole clinical manifestation was miliary infiltration of the lungs at the age of 3 [illegible] years.

KEY WORDS. Chronic granulomatous disease of childhood, disseminated BCG

Recently Esterly et al (5) described disseminated BCG in twin boys with a retrospectively probable diagnosis of chronic granulomatous disease of childhood (CGD) There are no other previous reports of BCG-immunized CGD-patients suffering from tuberculosis The present report describes a patient with CGD whose clinical picture has closely suggested the diagnosis of disseminated BCG although acid-fast organisms have never been found

The mode of genetic transmittance of CGD is not yet clear (1 3 4 14 18) This family is the first one to be reported having both an affected male and an affected female member throwing new light on the question of the mode of inheritance of CGD

Table 1 *Laboratory data on each admission of T M*

Date	Hemoglobin (g/100 ml)	Hematocrit (%)	Platelets (cells/μl)	WBC (cells/μl)	ESR (mm/h)
May 28 1966	8 9	34	284 000	11 400	36
May 10, 1967	9 8	31	170 000	4 900	38
March 3 1968	13.9	42	212 000	7 500	21
Sept 18 1968	1[illegible].1	40		6 300	29
Oct 1 1969	10.1	33		9 700	57
Jan 23 1970	10 6	3[illegible]	53 000	5 000	38
Oct [illegible] 1970	12 8			22 700	43
Aug [illegible] 1972	9 9	[illegible]	255 000	16 700	75

associated with poly-oligodactyly and skeletal (mainly vertebral) anomalies. *Acta Paediat Scand 60* 197 1971
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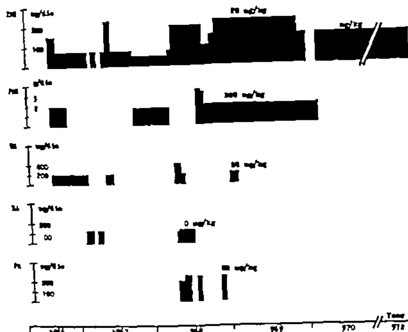


Fig. 2. Antituberculous medication of T. M. Isoniazid (INH), para-aminosalicylic acid (PAS), streptomycin (SM), ethionamide (EA), pyrazinamide (PA).

berculosis and found to be healthy. The girl had been given BCG inoculation in the neonatal period. The skin tuberculin tests were now negative. All cultures for acid-fast bacilli were negative. After 3 weeks of isoniazid therapy the chest X-ray film was normal. INH was discontinued after 26 months. One year later pulmonary changes, mainly fibrous ones, were again noted. They have persisted and progressed into massive general fibrosis and marked pleural thickenings, in spite of intermittent antibiotic therapy. Isoniazid was again started in Aug. 1972. The girl was then in a fairly good general condition. The liver and the spleen were not enlarged.

The NBT-test of this girl was also done *ad modum* Gifford & Malawista (6), which is not suitable for carrier diagnosis. Some formazan cells were formed but considerably fewer than in the control sample. The NBT-test of the healthy mother also showed scanty formazan cells. The father gave normal results, as did the third child, a healthy younger sister.

DISCUSSION

The case of T. M. is one of well-defined CGD. The patient had lymphadenitis at the time of the leucocyte bacteria-killing test and decreased bactericidal function has been found in many patients with bacterial infections (15). However, the percentage of formazan cells in the NBT-test has been reported to increase in patients with bacterial infections including active tuberculosis (9). In this case the NBT-test was repeatedly abnormal although bacterial infection was always present.

The diagnosis of disseminated BCG has been made mainly on clinical and morphological grounds. The initial histopathological picture of the lymph nodes and the liver was felt to be suggestive of tuberculosis. However, CGD granulomas often bear a striking resemblance to tuberculous granulomas (16). Pigmented lipid histiocytes often found in CGD (16) were not searched for. Miliary scarring in ocular fundi seems to be a common finding in CGD and does not point specifically to tuberculosis (10). The initially positive skin tuberculin tests support the diagnosis. The response of the patient to antituberculous therapy has been felt to be a strong point in favour of the diagnosis. In critical phases of the disease combined antituberculous therapy seemed to be dramatically effective, even life-saving. The slow deterioration of the general condition can be attributed to CGD.

There are reports of eight CGD-patients

CASE REPORT

Case 1

T M a male child born on Sept 8 1965 was referred to the Central Hospital of Tampere at the age of 8 months. The pregnancy and delivery had been normal and a BCG inoculation had been given in the hospital nursery On the initial admission the patient had two draining lesions on the left thigh at the sites of BCG vaccination There was hepatosplenomegaly and enlarged lymph nodes in the left groin The left choroid was full of inflammatory scars The chest X-ray film showed hilar adenopathy and peribronchial infiltration of the lungs An immunoglobulin electrophoresis showed a slightly raised level of IgG The bone marrow aspirate was normal For further laboratory data see Table 1

Skin tests with tuberculin produced a 4×7 mm induration with 0 1 TU and a 7×12 mm induration with 1 TU strength in 72 hours Cultures of gastric washings urine and spinal fluid were negative for acid-fast organisms Cultures of the draining vaccination sites were also negative Microscopic examination of lymph nodes from the neck and the left groin showed epithelioid cells Langhans giant cells and extensive caseous necroses Bacterial cultures were negative The child was started on antituberculous medication in July 1966 After 4 weeks of treatment the vaccination sites were healed

The patient received combined antituberculous therapy for 10 months. In May 1967 a liver biopsy was performed because of persistent hepatosplenomegaly It showed multiple small granulomas formed by epithelioid cells. On the Ziehl Neelsen stain small round particles were seen among the granulomas These were suspected to be morphologically atypical Calmette bacilli and isoniazid therapy was continued.

The patient was admitted to the hospital in March 1968 in severe respiratory distress The chest X-ray film revealed diffuse soft infiltration of the lungs suggestive of miliary pulmonary tuberculosis All cultures for acid-fast organisms were negative The respiratory distress subsided after two weeks of intensive therapy with streptomycin isoniazid ethionamide and pyrazinamide The lung infiltration cleared gradually in the course of 3½ months. PAS and INH therapy was continued

In Sept 1968 dense infiltration of the lungs was noted, suggesting pulmonary fibrosis This markedly diminished during steroid therapy which was given for 12 months at home In Oct 1969 the boy had cervical pre-auricular and inguinal adenitis *Staph aureus* and *Klebsiella* were cultured from the lesions. At this time a leucocyte bacteria killing ability test was performed *ad modum* Holmes et al (8). It showed impaired bactericidal capacity of the leucocytes, suggesting CGD (Fig. 1)

In Jan 1970 the patient was hospitalized for pneumonia. At this time hemolytic anemia was noted Splenectomy was performed in April 1970 The spleen weighed 760 g and a microscopic examination showed small granulomas formed by epithelioid cells A liver biopsy revealed the same type of granulomatosis. Before discharge in June 1970 the NBT-test was performed according to the method of Gifford & Malawista (6). It was quite abnormal—no formazan cells were formed—confirming the diagnosis of CGD

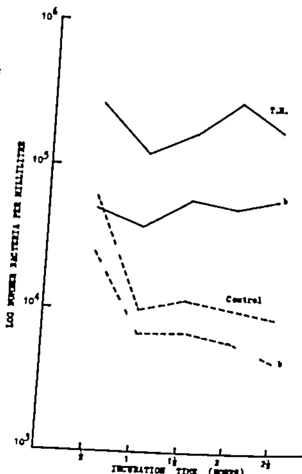


Fig 1 The rate of survival of *Staph aureus* in the presence of white blood cells Two experiments (*a* and *b*) are shown

In 1971 the boy was hospitalized for 4 months for septic fever Then a left perihilar consolidation was noted which resolved on isoniazid therapy alone The boy was under observation at the out-patient department for 14 months. He received multiple antibiotic drugs for respiratory infections. The NBT-test was twice repeated in this period and found to be abnormal.

In Aug. 1972 the patient was again admitted. His general condition had slowly grown worse with signs of cardiac hepatic respiratory and renal insufficiency He was still on isoniazid therapy at the age of 7 years. For the whole scheme of antituberculous medication, see Fig 2.

Case 2

T A M an older sister born on July 11 1964 was generally healthy until the age of 3 $^{7}/_{12}$ years when she became febrile The initial physical examination on admission in April 1968 was unrewarding. The chest X-ray film revealed soft miliary infiltration of the lungs. This occurred 2 years after antituberculous medication of the brother was started All possible contacts at home had earlier been thoroughly examined for tu

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CASE REPORT

FAMILIAL 4/22 TRANSLOCATION WITH PARTIAL TRISOMY FOR THE SHORT ARM OF CHROMOSOME 4 IN TWO SIBS

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From th Paediatric Clinic University of Padova, Padova Italy

ABSTRACT Sartori, E., Tenconi, R., Baccichetti, C. & Pujatti, G (Paediatric Clinic, University of Padova, Padova, Italy). Familial 4/22 translocation with partial trisomy for the short arm of chromosome 4 in two sibs. Acta Paediatr Scand 63: 631, 1974.—The further study of subjects, whose abnormal karyotype has been identified by means of the fine analysis of the chromatids with chromosome banding techniques, is necessary for valid comparison of the clinical patterns. In this paper chromosome banding was carried out by the reverse-staining Giemsa method in the 5 living members of a family with two sibs affected by the same abnormal phenotype. Severe mental retardation, stunted growth, peculiar facies, low-set ears, *turricephaly* and bilateral hip dislocation or hypoplasia were the main features. The mother and a normal sister had normal karyotype. The father presented a balanced translocation between the short arm of chromosome number 4 and the long arm of chromosome 22. The two malformed children were trisomic for a segment of the short arm of chromosome number 4 (4p14→4pter). The clinical picture observed in these malformed children is quite different from that noted in the only case reported of a child affected by a trisomy for the short arm of chromosome 4 identified by autoradiography alone. This may depend on the different amount of genetic material or on the different chromosome involved in the translocation.

KEY WORDS Mental subnormality partial trisomy of chromosome number 4, familial *chromosomal translocation 4/22, chromosome banding pattern*

In 1965 Wolf et al (9) and Hirschhorn et al (4) described a new clinically recognizable syndrome due to a partial deletion of the short arm of chromosome number 4 (4p-syndrome) This syndrome is characterized by low birth weight failure to thrive profound mental retardation microcephaly hypotonia, hypertelorism prominent glabella, cleft lip and/or cleft palate and other anomalies

All reported cases of the 4p-syndrome have been sporadic occurrences within unrelated families

In 1973 Schinzel & Schmid (8) described a family with the father as carrier of a balanced translocation between the short arm of chromosome number 4 and the long arm of chromosome 18 and an unbalanced child as trisomic for the segment of the short arm of chromosome 4

In this paper we would like to present a study of a family with a translocation between the short arm of chromosome 4 and the long arm of chromosome 22 in the father and in two sibs a trisomy for a recognized segment of the short arm of chromosome 4 The identification of this familial translocation was made by the reverse-staining Giemsa method (R-bands) (2)

who were given antituberculous therapy on the basis of the clinical and the morphological picture the cultures for tb-bacilli being negative (2 11 12 13 17) One of these patients (12) had been immunized with BCG vaccine In some cases (11 13 17) a favourable effect was noted Esterly et al (5) describe cases of disseminated BCG in twin boys with probable CGD Cultures for acid fast bacilli were positive The disease progressed rapidly and the patients did not respond to antituberculous medication There are two reports (1 7) of CGD patients who received BCG vaccination without apparent untowards effects

T A M is one of the few carrier females reported displaying possible clinical manifestations of CGD This is also the first family described to have both a male and a female CGD patient although this situation has been anticipated on theoretical grounds (3 4) Several modes of inheritance have been proposed X linked recessive (18) partially sex linked recessive (14) autosomal recessive with sex modification (3) or autosomal dominant with variable expressivity (1) This family would well fit into the mode of X linked recessive inheritance with the Lyon effect causing clinical signs in this female carrier T A M

Addendum

T M died after 4 weeks of septic fever at the age of 8 years The autopsy showed granulomas and pigmented lipid histiocytes in various organs. T A M died suddenly at home at the age of 8 ⁸/₁₂ years The autopsy revealed pigmented lipid histiocytes in various organs but no granulomas Both had extensive fibrous pneumonitis.

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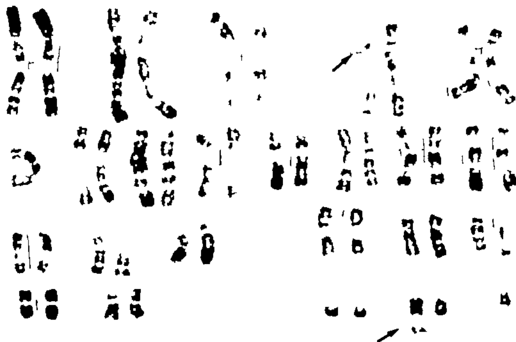


Fig. 4. The karyotype of the father. The arrows outline the translocated chromosomes.

were present. The head was small with a flat occiput. The face (Fig. 2) did not resemble that of the parents or her healthy sister at all but presented bushy eyebrows and long eyelashes, divergent strabismus, large and depressed nose bridge and an elongated philtrum. The mouth was large with a high arched palate and malaligned teeth. The ears were low-set, large and prominent. The hands showed bilateral clinodactyly of the 5th finger; the 1st, 2nd and 3rd fingertips of both hands were swollen and red with partially wasted nails due to continuous sucking. The feet showed bilateral hallux valgus with overlapping 2nd digit.

X-ray examination showed turricephaly, abnormal length of lumbar vertebral bodies and bilateral dislocation of the hip. The bone age was markedly retarded (corresponding to 18 months). Findings on intravenous pyelography were normal.

II-5. At 21 months the child weighed 8 kg, with length of 73 cm and head circumference of 41.5 cm. The child could not sit nor verbalize and it was impossible to establish any communication. On the Brunet Lezine test the D.Q. was 19. Slight generalized muscular hypertonia was present with tendency to flexion of the limbs and poor motility. Deep and superficial reflexes could not be elicited. Nystagmoid jerks were present only on extreme left lateralized gaze. The head was small with ... flat occiput. The facial features were strikingly similar to those above described for his sister (Fig. 3). The hands and feet also showed the same malformations.

X-ray examination showed turricephaly, bilateral hypoplasia of the hip. The bone age was markedly retarded (corresponding to 1 months). Intravenous pyelography was normal.

Routine laboratory data of both children were within normal limits and included complete blood count, urine analyses, blood glucose level, serum electrolytes, serum proteins and immunoglobulins, serum uric acid, blood urea nitrogen and ammonia concentration, serum protein-bound iodine, urine screening for aminoacids and mucopolysaccharides, E.E.G. and E.C.G.

Dermatoglyphs were examined in both parents, in the normal sister and in both affected children and revealed no abnormalities.

Cytogenetic Study

Chromosomal preparations obtained by usual technique from peripheral blood leucocyte cultures were treated with heat and Giemsa stain (2). The following members of the family were examined.

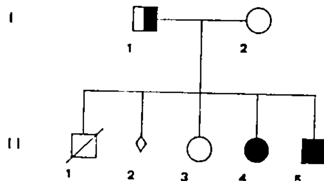


Fig. 1 Pedigree of family with 4/22 translocation ○ normal chromosomes ◨ balanced translocation ●■ unbalanced translocation ▨ stillbirth ◊ spontaneous abortion

Family History

No cases of malformation or mental retardation were recorded among the relatives The father born in 1935 and the mother born in 1941 were phenotypically normal and healthy

The mother presented a total of 5 pregnancies (Fig. 1)

The first full-term pregnancy in 1965 resulted in a stillborn macerated male of 2 7 kg. The 2nd pregnancy in 1966 resulted in an unexplained abortion at the second month In 1967 the 3rd pregnancy resulted in the delivery of a normal 3 1 kg female who is alive and healthy





Fig. 3 The patient II–5 at 21 month of age.



Fig. 2 The patient II–4 at 5 years of age.

The 4th pregnancy in 1968 resulted in the delivery of a 7 kg female and the 5th in 1971 of a 2.8 kg male Both presented peculiar facies low-set ears and bilateral clinodactyly of the 5th finger of each hand The neonatal period was uneventful for both, but growth was stunted and a severe mental retardation was noted in the first months of life At 9 months, the female child (II–4) was recognized to be affected by congenital bilateral hip dislocation and a corrective cast was applied for 2 months.

In 1972 they were admitted to our Clinic for further evaluation

Clinical Findings

II–4 At 5 years the child weighed 8 9 kg with a length of 80 cm and head circumference of 42.5 cm. The child was unable to sit and unable to speak and it was impossible to establish any communicative response from her On the Brunet Lezine test the D Q was <10 Discrete generalized muscular hypertonia was present with moderate flexion of the limbs and poor motility Deep and superficial reflexes could not be elicited Nystagmoid jerks on extreme lateralized gaze

The father phenotypically normal is balanced The unbalanced children may be trisomic for the segment 4p14→4pter if the breakage occurred just at the terminal of the long arm of chromosome 22 or less probably trisomic for the segment 4p14→4pter and monosomic for the segment 22q13→22qter if the breakage occurred at the terminal band of the long arm of chromosome 22.

Of the possible zygotes resulting from mating of a normal mother and a father who is carrier of this type of translocation we observed only two one normal and two trisomic but it is possible that the stillborn and the miscarriage were chromosomically unbalanced

One point we would like to stress is the striking similarity of the clinical pattern chiefly the facial features presented by the affected children

Varying clinical pictures have been observed in partial trisomy or monosomy of the B group chromosomes due to an unfavorable segregation in families with transmitted reciprocal translocation (1) (6) (5) (3) In most cases chromosome number 5 was involved only in one instance (3) was it doubtful whether the chromosome involved was number 4 or 5 As far as we know only Schinzel & Schmid (8) reported a partial trisomy 4p chromosome 4 being identified by autoradiography This was in a child whose father was carrier of a balanced translocation between the short arm of this chromosome and the long arm of chromosome 18 However the clinical pattern of this child was quite different from that observed in our cases This difference may depend on a different length of the 4p+ or a partial monosomy of chromosome number 18 (which is not possible to exclude by autoradiography alone) or finally on the different position of the translocated segment on chromosomes 18 or 22

ACKNOWLEDGEMENTS

We wish to thank Dr B Dallapiccola for his analysis of the dermatoglyphs and Dr L. D De Frank for revision of the English language.

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Fig 5 Partial karyotype of II–4 (*a*) and of II–5 (*b*) The arrow indicates the abnormal chromosome

I–1 In 28 well spread cells there was a male karyotype 46 XY and in all cells a trans location between two chromosomes was pres ent (*a*) Only one chromosome number 4 was recognized as being normal by means of the typical R-bands The other presented the long arm with identical band patterns but the short arm was deleted and presented only one dark band near the centromere (*b*) Only one chromosome number 22 was normal The other presented a four-bands segment at the end of the long arm (2 light and 2 dark alternately) (Fig 4) Interpretation balanced translocation between the short arm of chro mosome number 4 and the long arm of chromosome number 22 According to the inter national chromosome nomenclature Paris Conference 1971 (7) the karyotype of the father was 46 XY t(4,22) (p14 q13)

I–2 46 XX normal

II–3 46 XX normal

II–4 24 well spread cells were examined A female karyotype 46 XX was found with two normal number 4 chromosomes and only one normal 22 chromosome number the other being similar to the abnormal chromosome 22 observed in the father (Fig. 5 *a*) The karyotype was then 46 XX −22 +der(22) t(4,22) (p14 q13)

II–5 27 well-spread cells were examined A male karyotype 46 XY was found with the same abnormality observed in II–4 (Fig. 5 *b*)

In conclusion II–4 and II–5 were tri somic for a segment of the short arm of chromosome number 4 (4p14→4pter)

DISCUSSION

The genetic disorder we are dealing with is a familial translocation between the short arm of chromosome 4 and the long arm of chromosome 22

two other variables viz. frequency and rate of inspiration.

(c) *One year's experience in the treatment with CPAP in the Neonatal Department in Rigshospitalet Copenhagen*

During the period 3.8 1971–3 8 1972, a total of 69 neonates with respiratory insufficiency were treated with CPAP via endotracheal tube The two most frequent causes of respiratory insufficiency were hyaline membrane disease (45 patients of whom 27 survived) and severe rhesus immunization with ascites and hepato-splenomegaly (6 patients of whom 3 survived)

Hyaline membrane disease was diagnosed from the following criteria *Clinically* tachypnoea with a respiratory rate of more than 60/minute indrawing, grunting and central cyanosis in less than 40% oxygen *Radiologically* generalized finely-granular markings in the air-bronchogram *Pathological-anatomical* hyaline membranes and atelectatic pulmonary tissue The indications for treatment were the following (1) generalized cyanosis (2) apnoea and bradycardia or (3) arterial oxygen tension of <50 mm with 100% oxygen in the inspired air

Nine patients with hyaline membrane disease were treated primarily in a Bird respirator and CPAP treatment was employed here in the withdrawal phase Seven of these patients survived Thirty-six patients with the hyaline membrane disease were treated primarily with CPAP and 20 survived Only 12 patients survived with CPAP as sole form of treatment. Respirator therapy proved necessary in the remainder mainly on account of uncomplicated progressive respiratory insufficiency

Pa_{O_2} values prior to and after initiation of CPAP with an average pressure of 8 cm were available in 16 patients An average increase from 44–104 mm was observed which was greater in the group who survived than for those who subsequently died ($p<0.01$) P_{CO_2} and pH values were uninfluenced

Out of the patients with hyaline membrane disease who were primarily treated with CPAP three developed pneumothorax 14 atelectases and infiltrates and on autopsy hyaline membranes were demonstrated (100%) atelectases (100%) pneumonia (50%), pulmonary fibrosis (13%) subdural haemorrhage (13%) and kernicterus (13%)

On comparing with the results previously obtained in this department with respirator treatment CPAP appears to have reduced the mortality in patients with hyaline membrane disease from 51–32% when the birth weights are greater than 1500 g ($p<0.05$)

CPAP may be recommended as a simple and valuable adjuvant to conventional respirator treatment in patients with hyaline membrane disease As CPAP treatment alone was only sufficient in one-third of the cases it is not recommended unless it can be followed-up by respirator treatment in the department

DISCUSSION

D Benveniste· *Experience from the past four years in respirator treatment of neonates and premature infants with RDS and IRDS*

A special valve was employed (Brit J Anaesth *40* 464 1970) This valve has no movable parts weighs only 6 g, is 4 cm in length and has a dead-space of 0 5 ml and can be employed with all respirators The patient is able to breathe spontaneously at any time and independently of the respirator because the inspiration side of the valve is always in open connection with the atmosphere In order to ensure optimal moistening of the inspired air and to avoid condensation in the supply tube from the hygrotherm to the valve we have led warm water (37°C) from a pump via a baby feeding tube no 8 which is wound closely round the supply tube which connects the hygrotherm and the patient Employing this sys-

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PROCEEDINGS OF PAEDIATRIC SOCIETIES

DANISH PAEDIATRIC SOCIETY

Meeting October 13 1972

I Kamper P Bækgaard Birgit Peitersen P Marstrand Inge Tygstrup & B Friis Hansen *Artificial ventilation of neonates with respiratory distress*

(a) Respirator treatment of neonates with the respiratory distress syndrome (RDS) or the hyaline membrane syndrome

The etiology of the disease includes immaturity intra-uterine asphyxia low or absent surfactant defective alveolar expansion reduced alveolar ventilation increased respiratory exertion hypoxia acidosis (respiratory and metabolic) pulmonary vasoconstriction increased shunt circulatory shock increased capillary permeability exudation atelectasis and hyaline membranes The shunt may be extrapulmonary (foramen ovale and ductus arteriosus) and intrapulmonary

The first inspiration and filling of the alveoli with air are mentioned and the efforts and the pressures which are necessary under normal conditions and if surfactant is absent Finally normal respiratory volume vital capacity and the functional residual capacity of the lungs are mentioned On this basis the criteria for establishing the diagnosis of RDS are outlined and the indications for respirator treatment

RDS occurs in approximately 20% of premature infants and the mortality is approximately 50% Both the incidence and the mortality increase with decrease in the birth weight and gestational age

The effect of respirator treatment depends partly upon the fact that the respirator supplies the necessary quantities of air to the lungs when the respiratory movements are inadequate By varying the pressure volume, inspiratory phase and expiratory phase it is also possible to achieve and increase the functional residual capacity which counteracts formation of atelectasis and pulmonary oedema. The maximal pressure and in particular the duration of the average pressure have however an inhibiting effect on the pulmonary capillary circulation CPAP treatment is a rational form of treatment of RDS as the positive pressure counteracts pulmonary oedema and increases the functional residual capacity

(b) *The technique of continuous positive air way pressure treatment (CPAP)*

The CPAP system was introduced in June 1971 by Gregory In this form of treatment moistened inspiratory air is administered so that a slight positive pressure is established in the patient s airways corresponding to 8–12 cm water In this manner a more complete expansion of the lungs is obtained and this increases the functional residual capacity and reduces the alveolo-arterial oxygen gradient

A modification of Gregory s system is described which permits subsequent respirator therapy with minimal technical adjustments The system involves only two variables viz. oxygen percentage and pressure while ordinary respirators possess at least

two other variables viz. frequency and rate of inspiration.

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tem we have ventilated RDS patients for up to three weeks without formation of crusts or obstruction of the tube

R Stern H A Garfield & B Friis Hansen
The incidence of thromboses and significant infections in neonates treated with umbilical vein and arterial catheters

During the period 1 01 1971–1 01 1972 a material of 287 neonates were treated with catheters in the umbilical veins or arteries in the Neonatal Department Rigshospitalet Copenhagen Of these 181 survived

Growth of pathogenic bacteria was demonstrated in 23% on culture from blood during treatment or from the tip of the catheter after treatment despite prophylactic employment of 10 mg kanamycin per kg body weight administered in two doses daily Alpha-streptococci *Staphylococcus albus* and *Streptococcus faecalis* are considered to be non pathogenic The incidences of positive cultures were practically identical in the surviving patients and those who died after treatment for at least 96 hours (14% and 16% respectively) When treatment had been employed for more than 4 days a significantly greater incidence of 36% ($p<0.001$) was encountered (31% among the survivors as compared with 44% among those who died ($p>0.05$)) At autopsy in 106 patients thrombotic changes in the veins or arteries were encountered in 24% of patients treated for less than 4 days and 31% in those treated for more than 4 days ($p>0.05$) It is not possible at present to assess the prognostic significance of the thromboses

The incidences of both bacteriaemia and thrombosis were inversely proportional to the birth weights With birth weights <2500 g bacteriaemia and thromboses were encountered in 30 and 15% respectively while with birth weights >2500 g these findings were present in 16 and 6% respectively ($p<0.05$ and $=0.05$ respectively)

The indications for treatment with umbilical vein and artery catheters should be very strict and treatment should not exceed 3–4 days except on vital indications

DISCUSSION

J Mejer

During the period 1 7 1971–30 6 1972, 376 patients were admitted to our Neonatal Department Umbilical venous infusion was undertaken in 102 of these patients In fatal cases post mortem bacteriological investigation was undertaken Further culture was undertaken from the tip of the umbilical venous catheter when this was removed. Twenty-eight of the patients died Autopsy revealed thrombosis in the umbilical vein in one case only and thrombosis in the inferior vena cava in one case Positive cultures were obtained from post mortem bacteriological examination in 15 cases In 8 of these cases positive blood cultures (venule method) had been demonstrated previously Eleven of the infants had received chemotherapy In the cases in which positive post mortem bacteriological findings were present the infants had received infusion for an average of 4 days In cases in which the post mortem bacteriological findings were negative infusion had been administered for an average of 3 1/2 days In 34 cases bacteria were found on culture from the tip of the umbilical intravenous catheter Eight of these infants had received chemotherapy and 5 had positive blood cultures These infants had received infusions for over 4 days on an average Three of these infants had received chemotherapy In 29 cases no bacteria were found on culture from the tip of the umbilical venous catheter Six of these had received chemotherapy and one had a positive blood culture Infusion into the umbilical vein had been administered for an average of over 3 days in these children

If the infants in whom positive blood cultures were found and who received chemo-

therapy are excluded together with those in whom positive post-mortem bacteriological findings were present it is seen that 59 infants did not have generalized infections

If the infants who received chemotherapy are added to those with positive post mortem bacteriological findings it will be seen that 32 possibly suffered from sepsis and of these 14 had positive blood cultures and therefore probably had sepsis

F Urnn Knudsen & B Friis Hansen *The incidence of primary neonatal bacteriaemia in cases of primary rupture of the membranes discoloured amniotic fluid and pyrexia in the mothers*

A prospective investigation was undertaken from January 1970 to July 1972 with the object of investigating the incidence of primary neonatal bacteriaemia in cases with (1) primary rupture of the membranes >24 hours (2) discoloured and/or foul-smelling liquor and (3) maternal pyrexia prior to or during the delivery Immediately after delivery blood cultures were undertaken from (1) the infant via a catheter in the umbilical vein (2) from the umbilical veins at their placental attachment and (3) from the mother The investigation embraces 329 infants and 807 cultures (329 from infants 239 from the cord and 239 from the mothers)

In the infants cords and mothers growth was obtained on culture in 23% 9% and 4% respectively In 8 infants i e in 2.5% of the cases bacteriologically verified bacteriaemia defined as (1) growth of the same pathogenic bacteria in two different blood cultures or (2) growth on blood culture accompanied by severe clinical symptoms compatible with bacteriaemia In all 8 cases maternal blood cultures were negative i e haematogenous infection was not involved

The incidence of verified bacteriaemia in cases of discoloured liquor ($n=138$) was 0 7% with primary rupture of the membranes ($n=171$) 2% in maternal pyrexia ($n=12$) 8% and in cases of primary rupture of the membranes accompanied by maternal pyrexia ($n=8$) 50%

In cases of primary rupture of the membranes and/or maternal pyrexia the incidence of bacteriaemia could be correlated to the birth weight APGAR scoring and clinical symptoms during the first 24 hours of life in the following manner In birth weights >2 500 g, the incidence of bacteriaemia was 2% and in birth weights <2 500 g, 12% With APGAR scores of ≥6 the incidence of bacteriaemia was 0 7% and with APGAR scores of <6 20% When there were no symptoms during the first 24 hours of life the incidence of bacteriaemia with primary rupture of the membranes was 0 6% and when severe clinical symptoms were present 45%

In conclusion it may be stated that primary neonatal bacteriaemia occurred in the material investigated in cases with low birth weight low APGAR scoring and severe clinical symptoms compatible with bacteriaemia during the first 24 hours of life following deliveries complicated by primary rupture of the membranes and/or maternal pyrexia

H Sardemann & Inge Tygstrup *Cutaneous haemangiomatosis and prolonged jaundice*

Two patients are described neither of whom were predisposed to vascular malformations or jaundice and in both of whom the pregnancy had run a normal course and delivery and birth weights were normal Where both patients were concerned haemangiomata as large as walnuts were present on the foetal surface of the placentae and numerous small cutaneous haemangiomata were present in the skin at birth These were observed to increase in size and number during the first two weeks of life and then to regress spontaneously for some months from the fourth to fifth weeks of life

The patients were admitted for investiga

tion on account of severe prolonged jaundice of occlusive type with maximal serum bilirubin values during the second to third weeks of life (approx 260 μmol/l) The serum bilirubin values subsequently decreased parallel with the spontaneous regression of the cutaneous haemangiomata The bilirubin was mainly conjugated

Other laboratory findings revealed moderate increase in alanine amino-transferase with subsequent decrease to normal values Alkaline phosphatases were raised in one of the patients but returned to normal Investigations for metabolic disease infective or haemolytic conditions were negative Investigation of the urine for bile pigments and urobilin suggested occlusive jaundice

Cases with obstructive symptoms caused by haemangiomata have been described previously but the symptom combination of cutaneous haemangiomata and prolonged jaundice has not been described previously

It is concluded that in these two patients with similar case histories haemangiomata in the portal region have been present and have obstructed the biliary passages Following spontaneous regression of the vascular tumours bile flow has become normalized

J Ramsøe Jacobsen *Congenital heart disease in neonates*

The problems involved in newly born infants with symptom producing congenital heart disease are illustrated by a series of 18 infants in whom cardiac catheterization was undertaken during the first week of life Thirteen of the patients were examined during the first 48 hours and the investigations were frequently undertaken as emergencies Only 4 of the infants survived at the time of writing between 5 months and 3 years of age Seven infants died during the first day of life and 3 during the second day and one after 10 days Three of the deaths occurred in connection with operation Finally 3 infants died between the ages of 2 and 6 months This illustrates primarily that severe malformations are concerned which produce cardiovascular symptoms immediately after birth

The diagnoses were

	Number	Survivors
Transposition of vessels	5	2
Hypoplastic left heart	4	0
Pulmonary artery stenosis	4	1
Tricuspid atresia	1	1
Common vascular trunk	1	0
Endocardial fibroelastosis	1	0
Single ventricle aortic atresia	1	0
Aortic coarctation	1	0

In all of the patients with complete transposition balloon septostomy was performed. Two patients died shortly afterwards despite this one at the age of 2 months following initial improvement Three operations were undertaken two pulmonary valvulotomies and one atrial septostomy following unsuccessful balloon septostomy All 3 patients died during operation or postoperatively

Even although the symptoms are severe and the condition frequently ends fatally in a short time and the operative mortality will inevitably be high operative intervention may however prove successful in some cases Cardiac catheterization should be undertaken as rapidly as possible in neonates with symptom-producing heart disease in order to establish the diagnosis and provide the basis for intervention The significance of simultaneous intensive medical therapy is emphasized the object being to maintain the best possible circulation until the diagnosis is established and the patient submitted to operation if this is possible This consists primarily of ensuring the best possible oxygen supply counteraction of acidosis and in cases of congestive heart failure digitalization and diuretic therapy

Meeting November 17 1972

Henning Andersen *Growth and growth factors*

Kristian F Hanssen *Somatotropin physiology plasma and urinary determination*

A short review of the physiology of human somatotropin (human growth hormone (HGH)) was given Attention was drawn to the species specificity of the hormone and the striking molecular similarity between somatotropin human placenta lactogen and human pituitary prolactin The complexity of the physiological effects of somatotropin was stressed with emphasis on its marked anabolic effect.

Radioimmunoassay of somatotropin was described It was stressed that radioimmunoassay principally determines the immunogenic and not the biological activity of a given hormone The most widely used test to evaluate somatotropin release insulin hypoglycemia, was described and our experience with this test in the diagnosis of hypopituitarism in children was mentioned. Finally our experience with determination of urinary somatotropin was surveyed Using the method published by us it has been shown that determination of urinary somatotropin might be an aid in the diagnosis of hypopituitarism in children Furthermore in acromegaly the urinary excretion of somatotropin is elevated also in those patients that have plasma somatotropin within the normal range

K W Kastrup *Somatomedin physiology and serum analyses*

The term Somatomedin (Sm) is employed to denote the factor or factors which mediates the effect of growth hormone (HGH) on the cells The content of Sm in the serum is determined by a modification of K. Hall's method by which incorporation of radioactive sulphate in foetal chicken cartilage cells is measured after incubation with the serum concerned Autoradiography demonstrates the intracellular activity after brief incubation. Following more prolonged incubation the activity is observed to be extracellular to a greater extent probably as an expression of synthesis of chondroitin sulphate

In patients with retardation of growth but with normal immunoreactive growth hormone (IRHGH) normal Sm values are encountered In patients with hypopituitary growth retardation, reduced Sm values are found and similarly low Sm values were encountered in a patient with retardation of growth and high IRHGH levels By means of the insulin tolerance test some patients were found with high IRHGH response and a late increase in Sm which was not observed in patients with hypopituitary inhibition of growth Following infusion of 2 mg HGH increase in Sm after some hours was observed both in normal and hypopituitary patients In patients with inhibition of growth and high IRHGH this increase was not observed An increase in plasma insulin was observed as a finding which accompanied the increase in Sm in the former but not in a patient with high IRHGH

Thus alterations in the Sm content in the serum were demonstrated in relation to transient alterations in the concentrations of IRHGH

Henning Andersen Inger Høyer Kristian F Hanssen & K. W Kastrup *Hypopituitary hypothalamic growth retardation diagnosis and treatment*

K W Kastrup Kristian F Hanssen Henning Andersen & Kirstine Hauge Kristensen *Retardation of growth with high growth hormone levels in blood and urine*

A boy aged 4 years whose parents were consanguinous was admitted on account of

retardation of growth which had been recognized at the age of 6 months

Clinically he presented the classical symptoms of hypophyseal growth retardation Excretion of IRHGH (immunoreactive human growth hormone) in the urine was found to be raised and similarly the plasma IRHGH values were found to increase to very high values during hypoglycaemia produced by insulin The characteristics in these patients were first described by Laron. Low Somatomedin content is encountered in these patients and this it not increased following infusion of HGH The changes may be due to lowered receptor sensitivity to HGH

Meeting January 19 1973

K Siersbæk Nielsen P Rogowski & J Molholm Hansen *Thyroid function in the newborn evaluated by micro methods*

Thyroid function in the newborn has been evaluated using two new micro-tests for determination of total plasma thyroxine and non protein-bound thyroxine (Tetralute® and Trilute®) 202 newborn full term infants were examined on their second day of life and the normal range (95% limits) for plasma thyroxine was found to be 9 2–26 0 μg per 100 ml Non protein-bound thyroxine was evaluated with a T_3 test (Trilute®) and normal values varied between 42 5–64 9% Free thyroxine index calculated as the product of the two above mentioned tests was found to vary between 510–1 378 arbitrary units The mean values of total thyroxine T_3-test and free thyroxine index were found to be increased compared with cord blood and adult mean values indicating a physiological thyroid hyperfunction in the neonatal period The thyroid function tests used in the present study are technically very simple and can be performed using a total of only 100 μl plasma Micro-thyroid function tests are suggested to be used whenever thyroid diseases in the newborn are suspected

H Haase *Toxocariasis Larvae from canine roundworm in man*

After some weeks in open air a larva can develop in the egg of toxocara canis (t c canine roundworm) If the egg is then ingested the larva can bore its way through the intestinal wall and be carried to the liver lungs and other organs The larva can only develop to the adult reproductive phase in the dog Since 1952 findings of larvae in the liver lungs retina skin myocardium colon muscles brain and cranial bones in man have been reported

The condition is observed most frequently in children under the age of 5 years who eat soil The complaints experienced are frequently recurrent symptoms from the lower respiratory passages slight anorexia, slight failure to thrive and possibly slight pyrexia. Monocular disturbances of sight and epilepsy have also been reported Hepatomegaly and pronounced eosinophilia are practically invariably encountered Raised IgG and IgE values are frequently observed X-ray of the thorax may reveal transient infiltrates

Diagnosis On account of the minute size of the larva biopsy from the liver and other organs will often yield negative results Cutaneous tests with toxocara canis antigen 1 1 000 have been shown to be positive in patients in whom larvae have been demonstrated The same cutaneous reaction was found to be positive in scarcely 2% out of 485 apparently healthy individuals near London The fluorescence antibody test persists for a briefer period than the cutaneous test

Treatment with steroids is symptomatic. Thiabendazol=Mintezol appears to be the hitherto most effective form of treatment

In the Department of Paediatrics in Odense Hospital we have had a girl aged 2 years with recurrent pronounced asthmatic bronchitis hepatomegaly and eosinophilia increasing to a maximum of 82 000/μl The cutaneous tests and toxocara canis antigen reactions were positive Open liver biopsy was negative IgE was normal during steroid therapy

Following treatment with thiabendazol the patient has been free from symptoms for a year but moderate eosinophilia persists.

J Reimers *Dislocation of the hip in cerebral palsy*

Erik Lykkegaard Nielsen *Pyknodysostosis A review and report of six cases*

Pyknodysostosis is a genetically conditioned syndrome consisting of dwarfing with normal intelligence generalized osteosclerosis patent fontanelles and cranial sutures blunt mandibular angle and absence of processus unguiculares The mode of heredity is autosomal recessive In 1962 the condition was distinguished from osteopetrosis but it has not been described as an independent syndrome in Denmark

Six cases are presented all of whom had characteristic case histories In addition, 5 of the patients had symptoms which have not hitherto been recognised in this condition. they all had respiratory symptoms of varying degrees tendency to vomit and long soft palates One of the patients died at the age of 22 months on account of vomiting and pulmonary asperation Autopsy revealed normal conditions in the respiratory passages The prognosis in pyknodysostosis is thus perhaps poorer than was previously presumed

The presence of progressive acro-osteolysis has been discussed previously In one of the patients in this material a slight increase of bony tissue in the distal phalanges was observed by means of repeated radiographic investigations while in another patient both breakdown and synthesis were observed in the processus unguiculares These conditions are not yet fully elucidated

Birgitte Friis *Recurrent pustulosis in children from Korea*

In Korean children adopted by Danish parents skin changes are sometimes observed on arrival in Denmark or shortly afterwards. The changes are suggestive of scabies but neither burrows nor mites can be demonstrated and treatment for scabies has no curative effect.

Nine cases of Korean children aged about 8 months are mentioned These had generalized itching, finely papular rashes accompanied by outbursts of vesicles and pustules of a few millimetres in diameter and localized particularly to the hands and feet. The generalized rash disappears rapidly while the outbreaks of pustules on the hands and feet show a tendency to recur with varying intensity for several months The condition is characterized by accompanying slight eosinophilia, peripheral adenitis and in occasional cases splenomegaly Culture from the pustules is negative in the majority of cases No evidence of fungal infection can be found Histological investigation reveals that the pustules are situated subcorneally and are accompanied by slight inflammatory changes

The clinical picture does not resemble bullous impetigo and systemic antibiotic treatment is without effect. The skin condition may be an allergic reaction to a parasitous but repeated examination of the faeces has not revealed parasites The children have pronounced hyperhydrosis which may be a contributory cause Scabies treatment has not had curative effect. Some children have become symptom free without scabies treatment Local application of sulphur

retardation of growth which had been re cognized at the age of 6 months

Clinically he presented the classical symptoms of hypophyseal growth retardation Excretion of IRHGH (immunoreactive human growth hormone) in the urine was found to be raised and similarly the plasma IRHGH values were found to increase to very high values during hypoglycaemia p duced by insulin The characteristics these patients were first described by Lar Low Somatomedin content is encountered these patients and this it not increased fo lowing infusion of HGH The changes ma be due to lowered receptor sensitivity t HGH

Meeting January 19 1973

K Siersbæk Nielsen P Rogowski & J Molholm Hansen *Thyroid function in the new born evaluated by micro methods*

Thyroid function in the newborn has been evaluated using two new micro-tests for determination of total plasma thyroxine and non protein-bound thyroxine (Tetralute® and Trilute®) 202 newborn full term infants were examined on their second day of life and the normal range (95% limits) for plasma thyroxine was found to be 9 2–26 0 μg per 100 ml Non protein bound thyroxine was evaluated with a T_3-test (Trilute®) and normal values varied between 42 5–64 9% Free thyroxine index calculated as the product of the two above-mentioned tests was found to vary between 510–1 378 arbitrary units The mean values of total thyroxine T_3-test and free thyroxine index were found to be increased compared with cord blood and adult mean values indicating a physiological thyroid hyperfunction in the neonatal period The thyroid function tests used in the present study are technically very simple and can be performed using a total of only 100 μl plasma Micro thyroid function tests are suggested to be used whenever thyroid diseases in the newborn are suspected

H Haase *Toxocariasis Larvae from canine roundworm in man*

After some weeks in open air a larva can develop in the egg of toxocara canis (t c canine roundworm) If the egg is then ingested the larva can bore its way through the intestinal wall and be carried to the liver lungs and other organs The larva can only develop to the adult reproductive phase in the dog Since 1952 findings of larvae in the liver lungs retina skin myocardium colon muscles brain and cranial bones in man have been reported

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Treatment with steroids is symptomatic Thiabendazol=Mintezol appears to be the hitherto most effective form of treatment

N Bloch Jespersen & T Marner *Socio-economical conditions in children with psychic diseases*

This investigation consists of a retrospective account of the social conditions in children admitted to the Department of Paediatrics in Glostrup Hospital during the period 1967–70 The material consists of 256 children with behavioural disturbances psychosomatic conditions or neuroses and a control group of 299 children with acute appendicitis or inguinal hernia.

Definite over-representation of children from the poorest social classes was encountered 44% of the children with psychiatric conditions originated from the lowest social groups as compared with 25% in the control group Similarly the occurrence of crowded housing conditions broken homes and mothers with full-time employment outside the homes were somewhat more frequent among the children with psychiatric conditions

It is concluded that prophyaxis for psychic illness in children must include improvement in the conditions of the lowest social classes as a factor of importance Where the upper social classes were concerned no definite differences between the two groups investigated could be demonstrated with the parameters employed

Marie Schultz Andersen F Becker-Christensen C Hertel & Karen Thomsen *The influence of social factors on childrens acute admission to hospital*

Meeting March 9 1973

Karine Bech *Morphology of the foetal suprarenal cortex Normal development and changes in disease in mother and infant*

Foetal suprarenal cortex consists of two zones only One of these the foetal zone disappears completely by involution after delivery while the other the permanent zone grows and undergoes differentiation into the three zones which characterize the suprarenal cortex in adults In order to elucidate the morphological and temporal conditions of involution a selected material of 562 suprarenal glands from foetuses and neonates were examined histologically and compared with the changes which may be seen in a series of diseases in pregnancy and in infants in order to determine whether these show pathological deviations

Involution may be characterized as a reactionless haemorrhagic necrosis of the foetal zone and it commences in the second day of life regardless of the gestational age In Rh-immunization (22 cases) massive areas of necrosis were encountered in the foetal suprarenal cortex and pathological accumulation of lipid These changes were unrelated to the degree of immunization of the mother but were related to the haemoglobin level in the infant In maternal diabetes (20 cases) and toxaemia of pregnancy (11 cases) the changes were not conclusively pathologically abnormal and were unrelated to the mother s clinical condition. In all three instances more pronounced changes were observed in cases of reduced oestriol excretion in the urine during pregnancy (a total of 26 cases)

Among eight children with Down s syndrome the suprarenal glands were found to be hypoplastic in five cases and among 23 infants with cystic fibrosis pathological changes were observed in 14 cases without any relation to the other clinical findings This suggests endocrine involvement in these diseases Occurrence of abnormally large cells in the foetal zone is termed adrenal cytomegaly and was encountered in 2.7% of the material but with considerable preponderance among twins triplets and infants with deformities The cause of this is unknown but it may be expression of malig

containing preparations has dried up the pustules but did not prevent new lesions from developing No itching skin conditions have been observed among the immediate family members during the course of the condition

Meeting February 9 1973

Jens Christoffersen Elsa Rafn Gertie Ricklefs & J Vesterdal *Children s reaction to hospitalization An investigation in children aged three months to three years* (*Preliminary communication*)

Out of 576 consecutive admissions to the department for children aged three months to three years in Glostrup Hospital 128 children were selected at random During hospitalization 34% of the children did not appear to adjust satisfactorily at all These children did not differ from the remainder as regards siblings or the daily care prior to admission 50% of the children between 12 and 24 months did not adjust during hospitalization During hospitalization 20% of the children were visited for 3–5 hours while 50% were visited for 1–3 hours daily All of the children were visited most frequently by their mothers but fathers participated more or less equally in the visits in more than half of the cases

Following discharge approximately 70% of the children were themselves again within the first week and approximately 45% at once The period of adjustment was longest for children between 18 and 36 months In 80% of cases the parents were of the opinion that the children had one or more forms of reaction to admission Of these 25% had exclusively negative reactions (conditions which worried the parents) such as clinging to their mothers fear of doctors altered sociability sleeping or eating problems In 23% on the other hand the parents considered that problems such as these had diminished while 32% experienced a mixture of these positive and the above mentioned negative reactions Negative reactions were twice as common in boys as in girls Periods of hospitalization of less than four days gave very few reactions while operation appeared to cause many negative reactions after discharge Longer duration of adjustment and greater incidence of clinging to mothers were observed among the children who were visited for more than 3 hours daily This may perhaps be explained by the fact that a particularly close contact has existed between these children and their mothers (parents) prior to admission and/or that these mothers have been particularly observant of changes In the long run these children have probably greater chances for overcoming the sequelae of admission because they can obtain the attention which they need

T Marner & N Bloch Jespersen *Psychic diseases in a paediatric department*

During the period 1967 to 1970 328 children were admitted for psychological investigation in the Department of Paediatrics in Glostrup Hospital The majority were aged 6–9 years of age Three fourths of the children has psychosomatic disorders or behaviour disturbances 10% of the children examined had simultaneously serious somatic disorders The therapeutic proposal most frequently employed during the period of investigation was sojourn away from home most often in a Christmas Seal Home Psychotherapeutic contact was only offered in only a minority of cases

It appears reasonable to propose increased therapeutic activity in future including e g out patient family interviews in co-operation with the consultant in child psychiatry attached to the department

Our patient was a newly born boy whose mother developed increasing virilization during the 2 months prior to delivery The infant was delivered approximately 2 weeks before term by caesarean section and a large arrhenoblastoma was found at operation in the mother

The infant presented signs of slight virilization with growth of hair which was somewhat more marked than ordinary lanugo This abnormal growth of hair disappeared during the first weeks He had no deformities and, in particular the genitalia were normal In order to illustrate further how the abnormal maternal hormones which were probably primarily androgens influenced the infant s own steroid synthesis a series of investigations of steroid metabolites in the urine were investigated In 24-hour specimens of urine collected when the infant was 4 days old and 1 month old were found an increased excretion of completely foreign metabolites in addition to the normal cortisol and corticosterone metabolites and certain normally occurring unknown steroids Among the 17 KS also great quantities of foreign compounds were demonstrated. A possible enzymatic defect could not be determined.

When the infant was 2 1/2 months of age many unknown steroids were still present in the urine Simultaneously however increased excretion of pregnandiol and pregnantriol could now be demonstrated suggesting a defect in the 21-hydroxylase system By means of *in vitro* experiments it has proved possible to inhibit just this enzyme with androgens e.g. DHA It may be conceived that the raised androgen content in the maternal blood may have caused such a disturbance in the infant s enzyme system in foetal life

Investigation during the subsequent months showed a gradual return to normal of the steroid metabolites and by the time the boy was 6 months of age normal steroid synthesis had been established

Jens Christofferson *Cushing s syndrome in a child with a malignant suprarenal tumour*

The patient was a girl aged 2 years 4 months old For 4 months prior to admission, signs of virilization had been observed On admission in addition a palpable abdominal tumour and raised blood pressure were observed No alterations in the serum electrolytes or blood glucose values were demonstrated The plasma cortisol urinary 17 KS and 17 KGS were all greatly raised Differentiated analysis of the steroid metabolites in the urine revealed that the greater part of the 17 KS was dehydroepiandrosterone (nearly 600 mg/24 hours) Massive excretions of delta 5-metabolites and corticoid metabolites were also observed At operation the tumour was shown to originate from the left suprarenal Invasion of the inferior vena cava had occurred and the child died during operation

G Fonseca. *Cushings syndrome caused by a benign tumour of the suprarenal cortex*

The patient was a girl who had previously been healthy Birth weight 3400 g, length 50 cm From the age of 1 year pubic growth had occurred and from approximately the age of 15 months stagnation of the growth in length had occurred. On admission (at 20 months) the child was found to have a typical Cushingoid appearance with adiposity moon face and lanugo hair growth The pubes corresponded to stage 2, slight hypertrophy of the clitoris was present but no breast development. The length was 76 cm (7 cm less than average) and weight 11 0 kg (2 kg above average)

The following laboratory investigations were undertaken. Cortisol in plasma. 76 µg% (greatly raised) testosterone in plasma 0.38 µg% (raised) 17 KS in urine 2.4 mg/24 hours (slightly raised) 17 KGS in urine 9 0 mg/24 hours (raised) By means of intravenous urography and tomography of

nant degeneration or caused by an unknown virus The changes are seen inter alia in the recently described Beckwith Wiedemann s syndrome and in congenital adrenal hypoplasia of cytomegalic type

Knud E Petersen *Methods of assessing the function of the suprarenal cortex particularly determination of rates of production*

A brief review is presented of the analyses of suprarenal cortical function which are relevant in paediatrics The principal differences in analyses in blood analyses in urine and determinations of rates of production are emphasized The clinical condition of the patient and the circumstances at the time of analyses should be accurately known The analyses should preferably be carried out under basic conditions and during stimulation (or suppression) It is particularly important that the paediatrician knows the extent to which the analyses and loading tests which are employed in adults can be employed in children In many fields normal values for the various ages are not available When biosynthetic defects are concerned the specificity of the analysis employed may be decisive for the correct assessment

The method employed in determination of the rate of production (secretion rate) for suprarenal cortical hormones is reviewed Following intravenous injection of radioactive hormone one or more of the metabolites of the hormone are isolated from a 24-hour specimen of urine the specific activity of these is determined and the endogenic production is calculated from a dilution principle The method may be employed with considerable accuracy and under certain conditions and may be employed as a measure of production. The results of some typical analysis (under basic conditions and after stimulation) are presented

Meta Damkjær Nielsen & F Becker-Christensen *Determination of corticoid metabolites in the urine in children*

Research into the changes which occur in corticosteroid synthesis and metabolism after birth presents a vey interesting problem in paediatric endocrinology These changes are due to the structural changes in the foetal suprarenal to the neonatal suprarenal and thus alterations in the enzyme systems which contribute to actual synthesis of cortisol Although a number of these changes are known a series of unsolved problems still exist as regards the time-intervals for these changes the mechanism which release them and the physiological implications.

By means of a paper chromatographic method it has been possible to separate and determine the excretion of a greater number of metabolites of cortisol corticosterone and aldosterone in the urine and the precursors of these in steroid synthesis in children in various age groups The authors have found a great number of unknown steroid compounds in infants during the first three months of life in addition to the normal corticoid metabolites Other investigations have yielded similar results In particular 16-hydroxylated compounds could be demonstrated within the first months

After approximately 3 months the steroid synthesis becomes normalized and by and large the same corticoid excretion pattern is found as in older children and adults

Henning Andersen & E Thamdrup *The excretion of 17 KS in a patient with congenital adrenal hyperplasia treated with p-pills*

F Becker-Christensen & Meta Damkjær Nielsen *Effect on the foetus of maternal virilization*

Our patient was a newly born boy whose mother developed increasing virilization during the 2 months prior to delivery The infant was delivered approximately 2 weeks before term by caesarean section and a large arrhenoblastoma was found at operation in the mother

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the suprarenal regions an opacity approximately 5×6 cm was observed at the upper pole of the right kidney The kidney was pressed downwards and the calyces were compressed There was no evidence of metastases in the spine The concentration of alkaline phosphatases in the serum were normal

At operation a well-defined solid tumour nearly as large as a tennis ball and situated between the diaphragm and the upper pole of the right kidney was removed There were no traces of normal suprarenal tissue Histological examination revealed an adenoma of the suprarenal cortex No evidence of malignancy could be found

Two months after operation the Cushingoid appearance was definitely less pronounced and 6 months after operation it had disappeared completely Length 83 5 cm (growth 7 5 cm) The concentrations of testosterone and cortisol in the plasma and the excretions of 17 KS and 17 KGS in the urine were normal

Knud E Petersen *Difficulties in the exact biochemical diagnosis in congenital adrenal hyperplasia in the neonatal period*

Like others we have accounted difficulties trying to establish the exact enzyme-defect in a neonate with congenital adrenal hyperplasia.

The infant a girl with virilized external genitalia was studied on the tenth day of life Studies of the excretion of metabolites in the urine showed a high excretion of 3β-hydroxy Δ5-steroids especially the 16-hydroxylated compounds Pregnanetriol excretion was not as dominant as usual in ordinary 21 hydroxylase deficiency—the total pattern of excretion suggesting a 3β-hydroxy steroid dehydrogenase defect

The excretion of abnormal steroids was completely suppressed during treatment with 30 mg cortison daily Studies in the infant around the age of 6 months showed a typical 21 hydroxylase defect

Peter Rasmussen *Addison s disease in a 7 year old boy*

Meeting April 27 1973

J Haahr & J Brochner Mortensen *Estimation of glomerular filtration after one injection of ^{51}Cr EDTA*

Erling Jensen *A case of congenital oligonefronic renal hypoplasia*

E Hasch *Ultrasound diagnosis of renal and urinary diseases in children*

On the basis of 150 ultrasound investigations of kidneys and urinary tracts in children aged from one day to 15 years this method is found to be suitable for demonstration of aplasia and hypoplasia of the kidney ectopic kidneys double kidney and the various stages of hydronephrosis It is possible by means of ultrasound to differentiate between solid and cystic swellings in the renal region Residual urine can be demonstrated and measured

It is not possible to assess the value of ultrasound investigation as compared with the methods hitherto employed but it appears to fulfil a need in paediatrics for an atraumatic painless and safe method of investigation of the kidneys and renal tracts

E Rosendal *Cyclophosphamide-treatment of nephrotic syndrome*

Birgit Peitersen N E Skakkebæk & Henning Andersen *Cyclophosphamide treatment of the nephrotic syndrome in children its effect and side-effects*

During the period 1968–1973 six children with the nephrotic syndrome were treated

with cyclophosphamide in Barnehospitalet på Fuglebakken. Three patients suffered from idiopathic nephrotic syndrome 2 patients had nephrosis following Schönlein Henoch s purpura and 1 patient had chronic glomerulonephritis All of the patients were treated with steroids for varying periods the maximum being ten years Cyclophosphamide treatment was initiated in cases in which steroid had no effect or where continued steroid therapy proved necessary for many years on account of recurrence on attempted withdrawal Treatment with cyclophosphamide had good effect in 2 of the 3 patients with idiopathic nephrotic syndrome In the third patient recurrences continued to occur In the 2 patients with Schönlein-Henoch s nephritis good effects were similarly obtained One of the patients however had a minor recurrence shortly after withdrawal of the therapy but has experienced nothing since In the patient with chronic glomerulonephritis neither the steroid nor the cyclophosphamide produced effects

Among the side-effects depression of the bone-marrow alopecia and dyspepsia were observed All of these were reversible In one patient, marked haematuria was observed and treatment was therefore withdrawn All of the patients showed normal growth in length during treatment with cyclophosphamide Two of the patients are still too young for any assessment of pubertal development and gonadal function. One boy underwent a normal puberty during cyclophosphamide treatment and one girl had a normal puberty after cessation of cyclophosphamide treatment and has normal menstruation. One of the patients a girl died at the age of 14 years without evidence of pubertal development and the ovaries were macroscopically infantile Finally one boy showed no signs of pubertal development at the age of 14 years

Biopsy was undertaken on the testis of the boy who underwent normal puberty during cyclophosphamide therapy The biopsy revealed complete absence of germinative cells and the testicular tubules contained Sertoli cells exclusively The Leydig cells appeared to resemble normal cells from an adult and were estimated to be present in normal numbers

Meeting May 12, 1973

Niels Juel Christensen. *Reduced plasma adrenaline concentration in children with idiopathic hypoglycaemia*

By means of a sensitive and accurate double-isotope technique the plasma adrenalin concentration was determined in the fasting state and during insulin hypoglycaemia in (*a*) 11 normal adults (*b*) 3 normal children and (c) 4 children with idiopathic hypoglycaemia (who were not hypersensitive to leucine)

The adults and the 3 normal children responded to administration of insulin with a marked increase in the adrenalin concentration in the plasma. In the 4 children with idiopathic hypoglycaemia, practically no increase occurred

Ole Mortensen. *Exanthema subitum*

A M Møllekær & E. Reske Nielsen. *Kinky Hair Disease*

This condition is a sex-linked recessive condition with cerebral degeneration and characterized by early progressive psychomotor retardation, seizures a tendency to hypothermia and peculiar hair The condition is probably caused by deficiency of copper which results in changes in the brain arteries bones and hair

H Bækgaard Laursen *The epidemiology of congenital heart disease*

A material of 219 patients admitted for investigation of congenital heart disease during

1971 were reviewed in view of distribution of the individual cardiac conditions The geographical distribution of the birthplaces is mentioned A few large representative groups of congenital heart disease have been analysed in view of familial predisposition distribution according to sex and birth weight etc

T Klinge *Haemangiomata in children*

Some disease entities resembling syndromes in character and with haemangiomata as significant components are presented and characteristic examples of haemangiomata are presented including two cases of liver haemangiomata which were diagnosed and followed-up by means of abdominal angiography

K Brostrom & Esper Mortensen *Haemolytic anaemia caused by haemoglobinopathy with unstable haemoglobin*

A six year-old girl is presented who had suffered from moderate haemolytic anaemia from the age of 3 months The etiology was elucidated recently and the condition proved to be a haemoglobinopathy Probably a new unstable variant of haemoglobin is involved

Knud E Petersen

Acta Pædiatr Scand 63 651–669 1974

PROCEEDINGS OF PAEDIATRIC SOCIETIES

EUROPEAN SOCIETY FOR PAEDIATRIC GASTROENTEROLOGY

6th Annual Meeting in Helsinki Aug. 29–30 1973

R. Lodinová, V Jouja & V Wagner (Prague) *Serum immunoglobulins and coproantibody formation in infants after artificial intestinal colonization with E. coli 083 and oral lysozyme administration*

After oral administration, the non-enteropathogenic strain E coli 083 was detected in the stools of colonized infants from the 2nd day and remained dominant up to 16 weeks Serum antibodies against E coli 083 were found 4 weeks after colonization. At 16 weeks the antibody level did not differ from that of bottle-fed controls although breast-fed controls still had a low titer IgG passively transferred from the mother decreased slowly but an increase from the 12th week was probably due to endogenous production. In breast-fed controls no decrease was noticed The IgA level rose gradually from the 4th week in all groups.

Colonization did not significantly influence serum immunoglobulin levels Coproantibodies were detected in colonized artifically fed infants at 4 weeks and the maximum level persisted to 16 weeks In breast fed colonized infants the coproantibody level was significantly higher between 2 and 6 weeks than in controls

IgM in low levels was detectable only in the artificially fed colonized group from the 4th to 16th weeks. The secretory IgA level in breast-fed colonized and control infants was high between 1 and 8 weeks. In bottle fed colonized infants the IgA increase started from the 4th week and was significantly higher in the 6th 12th and 16th week than in controls.

No IgG was found in stool filtrates of any group Artificial colonization induced formation of secretory IgA. Passive transfer masked this effect in the breast fed infants In bottle fed infants artifical colonization caused active formation.

Lysozyme feeding did not affect the IgG level up to 16 weeks but at 5 and 6 months it was even higher than in breast-fed infants IgM was not affected by lysozyme IgA was higher in lysozyme-fed infants from the 12th week than in controls and at 16 weeks and 5 and 6 months higher than in breast-fed infants

In breast-fed infants high IgA levels were found in stool filtrates from the 1st to the 12th week i.e during the time of breast feeding In lysozyme-fed infants secretory IgA was higher than in controls up to the 16th week. Stool filtrates of lysozyme-fed infants contained only traces of lysozyme.

Gastrointestinal infections were less frequent in lysozyme-fed infants than in controls.

J Rey (Paris) *The diagnostic criteria of cow s milk intolerance*

S Freier (Jerusalem) *Allergic mechanisms in intestinal cow s milk intolerance*

1 That heredity plays an important part in intestinal allergy is demonstrated by the following observations

1971 were reviewed in view of distribution of the individual cardiac conditions The geographical distribution of the birthplaces is mentioned A few large representative groups of congenital heart disease have been analysed in view of familial predisposition distribution according to sex and birth weight etc

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Knud E Petersen

Our gluten intolerance cases biopsied without any restrictive preliminary diet have all shown subtotal or total villous atrophy with only one exception (partial villous atrophy observed before becoming total)

Broadly partial villous atrophy in our opinion has the same relation to cow's milk protein intolerance as subtotal or total villous atrophy has to gluten intolerance In itself, this statement may help diagnosis However neither partial villous atrophy nor a weak positive lymphoblast transformation test are specific to primary cow's milk intolerance and can be found in serious and persistent enteropathies of quite different etiology

This does not reduce the importance of intolerance to cow's milk proteins among partial villous atrophies and their connection with the diet (introduction or suppression of milk) proves the relation of these changes to this sort of intolerance

J Jodl, Z. Lojda & J Hoffman (Prague): *Intolerance to cow's milk proteins in children*

During 1965–1972 we observed 43 children with intolerance to cow's milk proteins The first clinical symptoms were diarrhoea vomiting and failure to gain weight and appeared mostly between 6 weeks and 4 months of age depending on introduction of cow's milk. Absorption tests showed malabsorption. Sera of all patients were examined for antibodies against cow's milk proteins and gliadin Ouchterlony's immunodiffusion technique was used for detection of antibodies against alpha-lactalbumin beta lactoglobulin and casein We could usually demonstrate clinical sensitivity to beta-lactoglobulin and exceptionally (in two patients) to casein only Enterobiopsy examinations were carried out. A systematic histological histochemical and biochemical study showed various degrees of damage to the intestinal mucosa The activity of brush border enterokinase was lowered more frequently than in children with other types of malabsorption syndrome Improvements in clinical symptoms and absorptive function were found on elimination of cow's milk We used therapeutic Nutramigen and Pregestimil with very good effect. The mucosal damage persisted in about 20% of our patients in whom true coeliac sprue was later confirmed

S Cadranel P Rodesch M J Mozin & H Loeb (Brussels) *Pitfalls in treatment of cow's milk intolerance soy-milk intolerance*

Both acute and chronic gastrointestinal reactions of infants to cow's milk are well established The usual treatment of these patients is to substitute soy-milk for cow's milk. Recently even soy-milk has been reported to cause acute symptoms We report the cases of 3 female infants with acute or chronic soy-milk intolerance following cow's milk intolerance or allergy

In the first case a malabsorption syndrome due to cow's milk intolerance with partial villous atrophy at the age of 3 months was not improved by a soy-milk diet After successful parenteral nutrition, normalisation of the intestinal mucosa was observed When soy-milk was given again acute gastrointestinal symptoms and shock occurred

In the second case soy-milk, given initially for familial cow's milk allergy induced diarrhoea and failure to thrive On subsequent cow's milk diet, no improvement could be seen but eczema appeared. At the age of 5 weeks the child presented with shock, within one hour after a single feed with soy-milk. Skin tests for soya were positive in these 2 cases In the third case a malabsorption syndrome with subtotal villous atrophy developed on a soy-milk diet formula given for eczema on cow's milk diet. Clinical and histological improvement occurred on human milk diet.

(*a*) If one sibling is affected there is a 2 1 chance of a further infant being similarly affected

(*b*) If mothers of sick infants are challenged with cow s milk 17% will still show untoward reactions

(*c*) A family history of allergic disease can be elicited in 70% of infants with cow s milk allergy

2 Certain proteins by dint of their molecular structure are more likely to cause allergic reactions These allergens are glycoproteins with molecular weights of 20000 to 40000 and include beta lactoglobulin of milk and ovomucoid of eggs

3 Cow s milk has an excessively high protein content for the needs of the new born infant Whole cow s milk protein is incompletely digested and this results in prolonged contact with the intestinal mucosa and greater likelihood of allergic reactions

4 The newborn infant is without intestinal secretory IgA As this immunoglobulin is an antigenic barrier greater amounts of undigested protein may cross the intestinal epithelium and provoke allergic reactions We know that gastrointestinal allergy becomes less pronounced with age

5 Immediate type anaphylactoid reactions appear to play an important part in intestinal allergy This is borne out by the observations that in milk allergy

(*a*) the Prausnitz–Küstner reaction may be positive

(*b*) antibodies to milk belonging to immunoglobulin E are present

(*c*) patient s sera degranulate mast cells in the presence of the appropriate antigen (milk protein) and

(*d*) reactions can be prevented by chromoglycate (Intal) given orally

6 Toxic complex reactions with Arthus type pathology and reduction of complement may also be seen in G I allergy Cellular immunity has also been implicated Work done so far indicates promising areas for further investigations

A S McNeish (Birmingham) *The role of lactose in Cow s milk Intolerance*

The concept and clinical features of secondary disaccharidase deficiency are well known The relation between this condition and cow s milk protein intolerance (CMPI) is complex and may be difficult to unravel by elimination diets alone Liu et al Demonstrated that cow s milk protein especially beta lactoglobulin could produce a flattening of the blood lactose absorption curve in babies with recent CMPI who were on a diet free from cow s milk protein The author recently investigated 5 cases of CMPI (confirmed by evidence of complement activation after milk challenge) Lactose challenge did not cause adverse symptoms but there was evidence of impaired absorption the results correlated poorly with jejunal disaccharidase levels These findings agree with the data of Lubos et al.

It is important to differentiate evidence of lactose malabsorption mainly biochemical from symptomatic lactose intolerance Lactose malabsorption is usual in CMPI but frank lactose intolerance may be uncommon

J L Fontaine J Navarro & C Polonovski (Paris) *The intestinal biopsy in 20 cases of intolerance to cow s milk proteins in infancy*

Intestinal biopsy in 20 cases with a clinical diagnosis of intolerance to cow s milk proteins supported in 12 cases out of 18 by a positive lymphoblast transformation test (especially to lactoglobulin) revealed

(*a*) anomalies in 100%

(*b*) total or subtotal villous atrophy in 0%

(*c*) every step from only lymphoplasmocytic infiltration (1 case) through slight or moderate villous atrophy (17 cases) to partial or almost subtotal villous atrophy (2 cases)

immediate-type hypersensitivity (IgE) The role of the thymus-dependent lymphocytes has been investigated by studying, in mice and rats small intestinal lesions which resemble villous atrophy These were produced by allograft rejection and by helminth infestation of the small intestine and immunologically normal animals were compared with thymus-deprived animals (which had profoundly impaired cell-mediated immunity) The results show that it is difficult to produce villous atrophy in thymus-deprived animals and suggest that T-lymphocyte-mediated immune reactions may directly influence small intestinal epithelial cell kinetics with the production of a coeliac-like morphology

This work correlates well with reports that patients with cow s milk intolerance may have normal titres of antibody to cow s milk but have circulating lymphocytes which react with cow s milk antigens

J Jos & G Lenoir (Paris) *Application of the organ culture method to physio-pathological studies in coeliac disease*

The organ culture method that we reported previously (de Ritis and Jos, communication to the ESPGA meeting, Hamburg 1972) has been improved to obtain better preservation of the morphology and functional state of the intestinal mucosa maintained in culture for up to 48 hours This was achieved by adding NCTC 135 and bicarbonate and increasing the concentration of foetal calf serum in the culture medium Histological examination by serial sections histochemical and immuno-histochemical reactions as well as electron microscopy studies confirm the excellent preservation of the fine structure and physiological condition of cultured mucosal specimens Various peptic-tryptic (P T) or peptic-tryptic-chymotryptic (P T C) hydrolysates were prepared from gliadin casein and human albumin and tested on biopsies obtained from normal controls and from children with coeliac disease These experiments showed that the different hydrolysates added to the children medium exert a non-specific cytotoxic effect on flat mucosae obtained from untreated coeliac children and from patients in relapse. No such harmful effect was seen in biopsies from other patients or from normal controls Several experiments were also performed on restored biopsies from treated coeliac children taken during a few days before and after gluten challenge before gluten challenge P T or P T C. digest of gliadin whether autoclaved or not, was slightly or not at all toxic to the epithelial cells of the cultured specimens while after gluten challenge these gliadin digests seemed to exert a specific noxious effect on the cultured biopsies which was not observed with the casein or albumin hydrolysate. If further experiments confirm these results the organ culture system may be valuable in tests on the toxicity of various gliadin hydrolysates or protein fractions when biopsies from coeliacs are obtained from treated patients and after a short gluten challenge

Margot Shiner (London) *A comparison of the ultrastructural appearances of reticulin endothelium and inflammatory cells of the jejunal mucosa in children with normal mucosae untreated coeliac disease and protein-energy malnutrition*

Immunological reactions local or general appear to play a prominent part in both coeliac disease and protein-energy malnutrition In untreated coeliac disease our histological and fine structural examination of the jejunal mucosa showed an increased infiltration of inflammatory cells (immunocytes) an increase in the reticulum and an endothelial hypertrophy This was coupled with increased immunological activity of the local plasma cells. In protein-energy malnutrition mucosal inflammatory cells may also be increased but we have observed that the immunocytes appear structurally inactive the

M Silverberg & M Davidson (New York) *Milk (bovine) protein gastrointestinal hyper sensitivity associated with eosinophilic gastroenteropathy in children*

Milk proteins have been implicated as a cause of diarrhoea colitis anaemia steatorrhoea and protein exudation In many of these patients a common denominator is the finding of eosinophilic gastroenteropathy (EG) characterized by (1) diarrhoea or vomiting (2) peripheral eosinophilia and (3) eosinophilic infiltration in otherwise normal intestinal mucosa biopsies Fourteen infants under two years of age were diagnosed as EG of whom nine were males Seven infants presented with bloody diarrhoea and peripheral eosinophilia and were intolerant repeatedly to bovine milks for 4–28 months of follow-up Seven otherwise thriving infants 8–18 months of age presented with signs of allergic gastroenteropathy i e iron deficiency anaemia occult blood in the stool and excess protein losses and relatively mild GI complaints The anaemia excess stool protein losses and eosinophilia cleared with dietary bovine and wheat protein elimination Steatorrhoea occurred only with concurrent gluten-intolerance Three of the older infants showed additional unexplained specific profound clinical intolerance with the development of shock like states on ingestion of rice in two cases and chicken in one instance Antibodies to bovine proteins in sera and stool were absent No primary immunological abnormalities or changes in serum complement were noted Protein intolerances have persisted for 8–36 months of follow-up manifesting shock bloody diarrhoea or eosinophilia with measured protein challenges

E Rossipal & L Auböck (Graz) *Electron microscope studies on the morphological changes of the small intestinal mucosa in three infants with cow s milk intolerance*

During the last two years we have seen five infants with cow s milk intolerance at the Kinderklinik in Graz In three of them the lesions of the small intestinal mucosa were studied with the electron microscope

In the acute phase of cow s milk intolerance electron micrographs revealed changes within the lamina propria similar to those seen in the acute stage of coeliac disease infiltration of highly active plasma cells and numerous lymphocytes The capillary endothelium showed swelling and destruction of the cell interior with vacuolation of the cytoplasm The perivascular membrane was thickened As in coeliac disease LE dermatomyositis and Sjögren syndrome virus like structures could be seen within the vascular endothelium In CMI damage to enterocytes was more intense and severe than in coeliac disease The cells in the lower part of the crypts already showed distinct signs of a lesion At the maturation zone the cells had the appearance of the dying cells at the extrusion zone But the microvilli which fell apart in drop-like particles were less stunted There was also no increase in the density of the cell membrane and no lysosomes filled with cellular debris could be found as in the extrusion zone

E M indicated that recovery from the ultrastructural changes in CMI is quicker than in coeliac disease In CMI many areas seem to be left which house enterocytes and still show ultrastructural damage However the degree of this damage appeared to be definitely less than in treated coeliac disease

Anne Ferguson (Glasgow) *Role of the lymphocyte in the pathogenesis of villous atrophy*

Villous atrophy of cow s milk intolerance and coeliac disease may be the result of immunological reactions in the wall of the small intestine—lymphocyte-mediated antibody-mediated (IgG IgA or IgM) or due to

disease including malabsorption osteogenesis imperfecta and congenital cyanotic heart disease In each child I[125] photon absorptiometry of the distal third of the left radius with a Cameron bone mineral analyser was performed, and X-ray pictures were taken of the left hand and of both tibial diaphyses The cortical thickness of the metacarpals and of the tibiae was measured with a micrometric magnifying glass Excellent correlation was found between direct mineralometry of the radius and the cortical thickness of the metacarpals ($r=0.89$) both in healthy controls and in sick children with reduced bone mass The correlation between mineralometry and tibial cortical thickness was lower ($r=0.78$) There was no correlation with the calculated cortical index

In the light of these findings metacarpal thickness was measured from X-ray pictures of the hands of about 200 unselected children (consecutive cases of untreated coeliac disease) aged 5 months to 16 years All these patients showed a flat duodeno-jejunal mucosa and responded well to gluten-free diet. In most of these patients the cortical thickness of the metacarpals was reduced below the 5th percentile This was especially evident in children over 18 months old In some cases this finding was the only sign of metabolic disorders and led to further investigations including intestinal biopsy to confirm the diagnosis of coeliac disease This simple method therefore proves to be a valuable tool in the investigation of intestinal malabsorption

M Papuzinski & T Zalewski (Warsaw) *An attempt at defining the character of intestinal changes in acrodermatitis enteropatica and studies on the pathogenesis of this disease*

On the basis of observations of 6 cases of a.e carried on over many years and of test made the authors discuss the following clinical pathogenetic and therapeutic problems of a e

1 In the early period of the disease the most prominent and even fatal symptoms is chronic diarrhoea leading at times to extreme inanition.

2. This diarrhoea is not infectious It consists in damage to intestinal walls destruction of intestinal villi and inflammation (cellular infiltrations in intestinal walls) which leads to the malabsorption syndrome and to fermentative diarrhoea.

3 In the opinion of experienced physician, the basic treatment method at that stage enabling patients to keep alive and giving oxyquinoline preparations a chance to act, is parenteral feeding.

4 Our working hypothesis on the essence of the troubles causing the pathological changes is as follows we are dealing in these cases with a permanent genetically conditioned TRY metabolic defect—leading to occurrence of toxic breakdown products of TRY metabolism and to incorrect oligopeptides

5 The mechanism of the so far most effective and empirically tested treatment by means of oxyquinoline preparations consists probably in competitive substitution of these compounds into TRY metabolites

M Gracey (Perth)· *Enteric disease in young Australian aborigines*

Diarrhoea and malnutrition are major causes for the high morbidity and mortality of children in under-privileged communities

Clinical pathological and pathogenic features were investigated in malnourished Australian aboriginal and Indonesian children.

Over 2 years 251 young aboriginal children with diarrhoea were studied 40% were malnourished and 37% were anaemic Sugar intolerance occurred in 25% two had monosaccharide malabsorption. More than 50% had stool pathogens Thirteen patients died 9 of these had fatty livers typical of

reticulin is sparse and the endothelium shows swelling and deficiency in endoplasmic reticulum It is postulated that the structural changes may be associated with decreased immunological activity within the jejunal mucosa and that this may be part of a more general immunological deficiency reflected in the reported thymic atrophy and inability to mount a delayed hypersensitivity reaction in malnutrition

K Harms E D Albert & R Wank (Munich) *A study of HL A in lymphocytes of 53 patients with coeliac disease and their families*

Fifty three unrelated children with coeliac disease (C D) 100 of their parents 58 siblings or other relatives and 462 unrelated healthy controls were investigated for HL A antigens

Of the 53 patients 34 (64%) were positive for HL A 1 (first HL A locus) and 35 (66%) for HL A 8 (second HL A locus) Both frequencies differ significantly (p 0 0005) from those for the control group (HL-A 1 27% HL A 8 19%) For the other 23 HL A antigens investigated there was no significant difference between patients and controls These results confirm previous reports by Stokes et al and by Falchuck et al for adult C D

Family studies and segregation analyses gave additional information

1 In C D HL A1 and HL A8 are mostly inherited together as HL A 1-8 haplotypes

2 There is co-inheritance of HL A genes and C D the haplotype HL A 1-8 was observed more often among patients than among their healthy siblings where it was evenly distributed

3 C D is more closely linked with HL A 8 than with HL A 1

It can be assumed that a gene partly responsible for coeliac disease is located in close proximity to the second HL A locus Three siblings of our patients with identical HL A 8 had a normal jejunal mucosa Therefore at least one other factor (environmental and/or genetic) besides gluten must be combined with the genetic effect of the second HL A locus if it is to lead to the manifestation of C D

B McNicholl B Egan Mitchell & P Fottrell (Galway) *Short onset coeliac disease*

22 of 193 children (18 male) had symptoms for less than 8 weeks 7 less than 4 weeks Age was 3½ to 18½ months 17 under 12 months Duodeno-jejunal biopsy showed severe grade atrophy (III) in 16 moderate (II) in 6 (I is mild 0 is normal) Anorexia and weight-loss occurred in all vomiting in 18 diarrhoea in 17 (alternating with constipation in 4) Vomiting with normal stools in 1 occurred in 3 with normal stools in 1 Haemoglobin was over 10 g in 21 serum iron under 75 μg in 8 of 9 children faecal fats over 3 g/day in 6/13 Blood glucose increment after lactose was under 20 mg/100 ml in 13/15 xylose absorption was impaired in 7/11 but 3 were not having gluten Length and weight were over 26th centile in 13/20 and 1/22 respectively and over 50th centile in 7/20 and 2/22 respectively Six disaccharidases were markedly depressed in 10/10 children In 12 children mean serumglobulins were G 800 mg/100 ml and M 98 mg/100 ml

D Nusslé (Geneva) M Tschopp D Shmerling (Zürich) B Hadorn (Bern) G Delézé (Lausanne) & A Donath *The significance of reduction of bone mass in children with coeliac disease assessed by measurement of metacarpal cortical thickness*

The accuracy of the measurement of metacarpal cortical thickness according to Bornard (1968) for the assessment of bone mass in children was tested in 96 children aged 1 to 16 years 78 of them were healthy controls and 18 were children with chronic

nitrogen intake at a constant caloric intake a strict intercorrelation was found between the amounts of phosphorus calcium and nitrogen retained Metabolic accidents due to excessive protein and caloric intake are avoided by very gradual increase of these intakes initially and their restriction to 100 calories/kg and 400 mg nitrogen/kg with simultaneous adjustment of phosphorus and calcium Infectious complications are avoided by strict adherence to the rules of asepsis

H Chr Børresen (Oslo) *Intravenous feeding in paediatric surgery*

Total intravenous feeding of paediatric surgical patients has been used with increasing frequency in the period 1966–1973 in the Department of Paediatric Surgery Rikshospitalet, Oslo Of the 478 newborns referred to our department in the 4-year period 1969–1972 the condition in 112 was considered serious enough to warrant postoperative total intravenous feeding. 88 survived and recovered. In the same period only 3 were lost among 40 older infants put on intravenous feeding due to high postoperative complication risk.

Our standardized routine intravenous feeding programme based on L Aminofusin Pfrimmer and Intralipid Vitrum" will be presented The impact of total intravenous feeding on surgical technique as well as on convalescence and recovery of individual patients will be illustrated Multiple simultaneous balance data show that the intravenous feeding programme sustains normal growth in small infants even in the immediate postoperative period Reversal of severe protein calorie malnutrition has also been achieved In most cases intravenous feeding according to our methods can be carried out for several weeks in peripheral veins with the use of infusion pumps and disposable particle filters The associated excretory osmotic load on the kidneys does not significantly exceed the corresponding figure for breast feeding Monitoring of patients on intravenous feeding by assays of serum amino acid pattern ammonia and triglycerides was discussed The composition of a new solution specifically designed for intravenous feeding of prematures and other neonates was presented

C Ricour & S Balsan (Paris) *Calcium phosphorus and nitrogen retention in long-term parenteral nutrition in infants*

During the past 3 years 42 infants have been on long-term parenteral nutrition In 7 of these patients an important hypophosphataemia (P below 20 mg/l) associated to neurological symptoms in one case was observed without any significant change in serum Ca concentration. The purpose of the present study was. 1) to determine the amounts of i v calcium phosphorus and nitrogen necessary for the prevention of such accidents 2) to analyse the factor(s) responsible for phosphorus depletion during long-term parenteral nutrition. This investigation was done by stable balance technique under a constant caloric intake (100 Cal/kg/24 h) calcium phosphorus and nitrogen intakes were varied simultaneously or alternatively Ca from 25 to 60 mg/kg/24 h P 10 to 60 mg/kg/24 h and N 320 to 640 mg/kg/24 h The subjects studied included 9 infants Results are as follows. 1) Phosphorus balance is highly correlated with calcium nitrogen and caloric intakes 2) Injection of excessive amounts of protein and/or calcium intravenously may induce a state of phosphorus depletion probably secondary to cellular anabolism and 3) A caloric intake of 100 Cal/kg/24 h a calcium intake of 35 mg/kg/24 h a nitrogen intake of 400 mg/kg/24 h and a phosphorus intake of 45 mg/kg/24 h will prevent this mishap and promote satisfactory development of the child.

kwashiorkor Severe bacterial and fungal infections and parasitic infestations were common Similar features occurred in the Indonesian children in contrast to the relatively mild pattern of enteric infections in well nourished European children

Management is very difficult prolonged intravenous nutrition and sugar-restricted diets are usually needed Initial therapy with a carbohydrate free diet (CF 1 Nestlé) reduces diarrhoea and assists nutritional rehabilitation

Pathogenesis of diarrhoea in these and other malnourished children is complex Important factors include low living standards repeated exposure to infections intestinal mucosal damage and secondary disaccharidase deficiency Local and systemic immunoglobulin levels were normal in both groups yet gross bacterial contamination of the upper gut was common Some of the organisms isolated were shown to inhibit intestinal sugar transport *in vitro*

K Bozkowa E Misiurewicz (Warsaw) A Bardoń K Borowicz Z Grodzka & E Sendecka *Comparison of the results of small intestinal biopsy and clinical symptoms in children with malabsorption*

Small-intestinal biopsy was performed on children suspected of malabsorption syndrome Later histopathological changes were estimated as well as disaccharidase activities lactase sucrase and maltase (by Dahlqvist s method) Specimens of the mucosa were taken from the jejunum some centimetres beyond the duodeno-jejunal flexure Furthermore in all cases the *d* xylose absorption test oral loading with lactose and sucrose and measurement of fat in faeces were carried out

The authors attempt to estimate the extent to which the clinical symptoms and results of examinations of the function of small bowel mucosa correspond to the results of intestinal biopsy

The decrease of the disaccharidase activities and degree of histopathological changes were more or less parallel In some cases on the basis of clinical manifestations and data obtained from the tests mentioned above we would have expected different results of biopsy—worse or better than were in fact observed

The communication was followed by a short discussion on the probable causes of the disordances observed

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From February 1970 to February 1973 we utilized 68 caval catheter (mean catheter life 60 days range 8 days to 7 months) in 50 children 42 aged from 15 days to 16 months and 8 aged from 5 to 14 years (25 cases of fistulas or subtotal resections of the small intestine 20 cases of severe malnutrition due to prolonged severe diarrhoea 5 cases each of acute haemorrhagic pancreatitis portal cavernoma and oesophageal perforation and 2 of severe renal failure)

Average water and electrolytic intakes per kg of body weight per day were water 120 ml sodium 3 mEq potassium 5 mEq magnesium 10 mg calcium 35 mg phosphorus 45 mg the nutrients given (per kg/day) were glucose 24 g and crystallized amino acids (200–400 mg nitrogen) vitamins and trace elements are added to the solution Once a week essential fatty acids were injected into a peripheral vein 14 children died 3 the first year because of vascular and infectious complications the other 11 because of irreversible conditions In the other 36 cases the results were very encouraging with a mean weight gain of 150 g per week and an increase in height of 2 to 3 cms per month Nitrogen retention always exceeded 40 per cent reaching 80 per cent with the lowest

nitrogen intake at a constant caloric intake a strict intercorrelation was found between the amounts of phosphorus calcium and nitrogen retained Metabolic accidents due to excessive protein and caloric intake are avoided by very gradual increase of these intakes initially and their restriction to 100 calories/kg and 400 mg nitrogen/kg, with simultaneous adjustment of phosphorus and calcium. Infectious complications are avoided by strict adherence to the rules of asepsis

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post-operative nutritional rehabilitation, such as following massive intestinal resection MCT reduce malabsorption and improve symptoms in patients with liver disease MCT are also beneficial in acquired or congenital lymphatic disorders such as intestinal lymphangiectasia. They have been reported to be useful with α-β-lipoproteinaemia. Preliminary results suggest MCT may be a useful source of calories in low-birth weight babies in tropical sprue and in children with protein-energy malnutrition. Despite such a formidable list it must be stressed that MCT are not a panacea for all forms of malabsorption. Indeed they do not cure the underlying conditions and should be considered rather as a useful source of calories in these situations

U Spahn & W Plenert (Jena) *Some biochemical aspects of obesity in children*

Since grossly obese children seem to be an inhomogeneous group regarding their metabolism we compared some biochemical findings obtained in 18 patients during one week of starvation Our preliminary results can be summarized as follows All these patients showed a marked rise in ketone bodies indicating that lipolysis was not impaired during five days of total fasting. Only half the patients however had serum FFA levels higher than 1.2 mval/l We therefore distinguished two groups of obese children. Group I was characterized by relatively low FFA levels accompanied by a closely correlated increase in ketone bodies (r=0.837) whereas in group II the increase in FFA was greater but there was no significant correlation with the ketone bodies (r=0.298). In group I the mean FFA value after starvation amounted to 0.844±0 287 mval/l and that of ketone bodies to 4 156±1.374 mMol/l. The corresponding data of group II were 1.800±0 447 mval/l FFA and 2.980±0 833 mMol/l ketone bodies (sum of serum acetoacetate and beta-hydroxybutyrate) No differences in serum glycerol could be established The increases of both cholesterol and triglycerides were higher in group I than in group II Interpreting these results we presume that in obese children biochemical differences may exist regarding the utilization of mobilized FFA during enhanced lipolysis Preferential uptake of FFA by the liver in some obese children causing enhanced synthesis of ketone bodies triglycerides and cholesterol is a possibility that has to be considered This hepatic channelling might depend on the serum levels of FFA However further investigations are needed before a definite decision can be reached

E. Signer G M Murphy S Edkins & C M Anderson (Birmingham) *The role of bile salts in the fat malabsorption of premature infants*

Premature infants exhibit a marked malabsorption of fat. Although the etiology of this malabsorption is unknown low bile acid concentrations have been reported in duodenal contents of premature (Lavy et al 1971) and full-term babies (Norman et al 1972) To date, there has been no report of the concentrations of bile acids obtained in the duodenum of premature infants in response to a milk feed The present study was therefore designed to provide this information and to assess its importance in relation to type of feed

Eighteen premature infants were studied. Nine were fed with human milk and nine with a modified cow's milk (Ostermilk) Subsequent to a 72-hour fat balance duodenal intubation was performed on the 14th day of life Total bile acids were determined in serial duodenal aspirates before and after a milk feed. Bile acid excretion in the faeces during a 72-hour period was also measured

Babies fed with human milk absorbed more fat (mean FAC=75%) than those receiving a cow's milk formula (mean FAC=56%).

P P F X Forget J Fernandes & P Haverkamp-Begemann (Rotterdam) *Enhancement of fat utilization during prolonged intravenous feeding*

An 8 year-old girl with severe underweight caused by anorexia nervosa was treated with total intravenous nutrition for 4 weeks During this period the intralipid dosage was increased stepwise the doses of Vamin® and glucose being kept constant The intralipid dosage was monitored by determination of the serum intralipid levels Fat utilization was investigated by intravenous fat intolerance tests and estimation of post heparin lipoprotein lipase activity of the plasma The intralipid elimination constant increased from 7% to 22%/min the L P L. activity increased from 50 to 300 μEq fatty acid/l/min These data enabled us to increase the intralipid dose from 3 g fat/kg/per day to 9 g fat/kg/per day without an increase of the blood triglyceride levels We may conclude that L P L is an inducible enzyme It is not clear which component of the hypercaloric intravenous regime causes this induction

B Persson & B S Lindblad (Stockholm) *Elemental diet in the rehabilitation of gastrointestinal disorders*

A commercially available elemental diet (Vivonex 100) was tried in the dietary rehabilitation of children with the diarrhoea malabsorption-undernutrition syndrome This was given in view of the reduced pancreatic enzyme excretion and atrophy of the surface jejunal epithelium with reduced dipeptide hydrolyse and disaccharidase activity resulting from undernutrition

At the Ethio-Swedish Paediatric Clinic in Addis Abeba 13 patients with the Kwashiorkor syndrome accepted the bottle with Vivonex earlier and showed less vomiting than 8 patients on a standard Casilan diet. A significant increase of mean haemoglobin concentration was seen only in the Vivonex group The high osmolarity of Vivonex (91 kcal% glucose 8 kcal% amino acids and 1 kcal% safflower oil=840 mOsm/l) might have contributed to the diarrhoea seen in some Vivonex cases A drop in FFA and β-OH butyrate may have been attributable to the very low fat content of the Vivonex preparation Eleven infants with severe marasmus and diarrhoea at the Jinah Postgraduate Medical Centre Karachi were given Vivonex during a 10-day initial rehabilitation trial—from the day after the necessary intravenous fluid and electrolyte treatment All infants improved and showed a considerable weight increase in contrast to the 30% mortality in a control group of 21 cases on the traditional high calorie-high protein diet

An elemental diet (with 50 kcal% sucrose 8 kcal% amino acids corresponding to the relative concentrations in human milk 4? kcal% corn oil and vitamins electrolytes and trace elements according to the known requirements of infants=510 mOsm/l) has been successfully used for 6 weeks in the rehabilitation of an infant with a short gut syndrome and for 6 months in a case of nonketotic hyperglycinaemia without any signs of nutritional deficiency

M Cracey (Perth) *The Clinical use of medium chain triglycerides (MCT)*

MCT are absorbed effectively in various disease states whereas the more usual dietary long-chain triglycerides are not They are therefore a useful caloric source in these situations In childhood MCT have been found useful in the following conditions In cystic fibrosis they reduce steatorrhoea and improve the stool pattern they are also a useful source of calories in patients recovering from infections and meconium ileus and may improve abdominal discomfort and rectal prolapse In coeliac disease MCT may be helpful in improving nutrition in severely affected patients Similarly they may assist in

post-operative nutritional rehabilitation such as following massive intestinal resection. MCT reduce malabsorption and improve symptoms in patients with liver disease MCT are also beneficial in acquired or congenital lymphatic disorders such as intestinal lymphangiectasia. They have been reported to be useful with α-β-lipoproteinaemia. Preliminary results suggest MCT may be a useful source of calories in low-birth-weight babies in tropical sprue and in children with protein-energy malnutrition. Despite such a formidable list it must be stressed that MCT are not a panacea for all forms of malabsorption. Indeed they do not cure the underlying conditions and should be considered rather as a useful source of calories in these situations

U Spahn & W Plenert (Jena) *Some biochemical aspects of obesity in children*

Since grossly obese children seem to be an inhomogeneous group regarding their metabolism we compared some biochemical findings obtained in 18 patients during one week of starvation. Our preliminary results can be summarized as follows All these patients showed a marked rise in ketone bodies indicating that lipolysis was not impaired during five days of total fasting. Only half the patients, however had serum FFA levels higher than 1.2 mval/l We therefore distinguished two groups of obese children. Group I was characterized by relatively low FFA levels accompanied by a closely correlated increase in ketone bodies ($r=0.837$) whereas in group II the increase in FFA was greater but there was no significant correlation with the ketone bodies ($r=0\ 298$) In group I the mean FFA value after starvation amounted to 0.844±0 287 mval/l and that of ketone bodies to 4 156±1 374 mMol/l. The corresponding data of group II were 1.800±0 442 mval/l FFA and 2.980±0.833 mMol/l ketone bodies (sum of serum acetoacetate and_beta-hydroxybutyrate). No differences in serum glycerol could be established The increases of both cholesterol and triglycerides were higher in group I than in group II Interpreting these results we presume that in obese children biochemical differences may exist regarding the utilization of mobilized FFA during enhanced lipolysis Preferential uptake of FFA by the liver in some obese children causing enhanced synthesis of ketone bodies triglycerides and cholesterol is a possibility that has to be considered This hepatic channelling might depend on the serum levels of FFA However further investigations are needed before a definite decision can be reached

E Signer G M Murphy S Edkins & C M Anderson (Birmingham) *The role of bile salts in the fat malabsorption of premature infants*

Premature infants exhibit a marked malabsorption of fat Although the etiology of this malabsorption is unknown low bile acid concentrations have been reported in duodenal contents of premature (Lavy et al 1971) and full-term babies (Norman et al 1972) To date there has been no report of the concentrations of bile acids obtained in the duodenum of premature infants in response to a milk feed The present study was therefore designed to provide this information and to assess its importance in relation to type of feed

Eighteen premature infants were studied Nine were fed with human milk and nine with a modified cow s milk (Ostermilk) Subsequent to a 72 hour fat balance duodenal intubation was performed on the 14th day of life Total bile acids were determined in serial duodenal aspirates before and after a milk feed. Bile acid excretion in the faeces during a 72-hour period was also measured

Babies fed with human milk absorbed more fat (mean FAC=75%) than those receiving a cow s milk formula (mean FAC=56%).

P P F X Forget J Fernandes & P Haverkamp-Begemann (Rotterdam) *Enhancement of fat utilization during prolonged intravenous feeding*

An 8 year-old girl with severe underweight caused by anorexia nervosa was treated with total intravenous nutrition for 4 weeks During this period the intralipid dosage was increased stepwise the doses of Vamin® and glucose being kept constant The intralipid dosage was monitored by determination of the serum intralipid levels Fat utilization was investigated by intravenous fat intolerance tests and estimation of post heparin lipoprotein lipase activity of the plasma The intralipid elimination constant increased from 7% to 22%/min the L P L. activity increased from 50 to 300 μEq fatty acid/l/min These data enabled us to increase the intralipid dose from 3 g fat/kg/per day to 9 g fat/kg/per day without an increase of the blood triglyceride levels We may conclude that L P L. is an inducible enzyme It is not clear which component of the hypercaloric intravenous regime causes this induction

B Persson & B S Lindblad (Stockholm) *Elemental diet in the rehabilitation of gastrointestinal disorders*

A commercially available elemental diet (Vivonex 100) was tried in the dietary rehabilitation of children with the diarrhoea malabsorption-undernutrition syndrome This was given in view of the reduced pancreatic enzyme excretion and atrophy of the surface jejunal epithelium with reduced dipeptide hydrolyse and disaccharidase activity resulting from undernutrition

At the Ethio-Swedish Paediatric Clinic in Addis Abeba 13 patients with the Kwashior kor syndrome accepted the bottle with Vivonex earlier and showed less vomiting than 8 patients on a standard Casilan diet. A significant increase of mean haemoglobin concentration was seen only in the Vivonex group The high osmolarity of Vivonex (91 kcal% glucose 8 kcal% amino acids and 1 kcal% safflower oil=840 mOsm/l) might have contributed to the diarrhoea seen in some Vivonex cases A drop in FFA and β-OH butyrate may have been attributable to the very low fat content of the Vivonex preparation Eleven infants with severe marasmus and diarrhoea at the Jinah Postgraduate Medical Centre Karachi, were given Vivonex during a 10-day initial rehabilitation trial—from the day after the necessary intravenous fluid and electrolyte treatment All infants improved and showed a considerable weight increase in contrast to the 30% mortality in a control group of 21 cases on the traditional high calorie-high protein diet

An elemental diet (with 50 kcal% sucrose, 8 kcal% amino acids corresponding to the relative concentrations in human milk 42 kcal% corn oil and vitamins electrolytes and trace elements according to the known requirements of infants=510 mOsm/l) has been successfully used for 6 weeks in the rehabilitation of an infant with a short gut syndrome and for 6 months in a case of nonketotic hyperglycinaemia without any signs of nutritional deficiency

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dren with active coeliac disease Enterokinase which is said to have the same subcellular localization as the disaccharidases is not significantly reduced in coeliac disease In some totally atrophic mucosae high activities of enterokinase were found It was suspected therefore that the subcellular localization of enterokinase was more complex than that of sucrase The enzyme was found in high concentrations in an almost pure preparation of human brush border membrane A second localization was also found. a fraction of the mucosal homogenate containing only small amounts of brush border but large amounts of endoplasmic reticulum had a disproportionately high content of enterokinase The enzyme occurred in a particulate and not fully active form To our knowledge this is the first report of an intracellular localization for an intestinal enzyme One wonders whether this intracellular form represents a precursor of the active brush border bound enterokinase

E. Eggermont & L. Rutgeerts (Leuven) *The effect of glucagon on mucosal enterokinase*

Enterokinase a brush border enzyme of the duodenal mucosa, is released into the luminal content by the action of several agents such as bile salts pancreatic proteases and/or hormones. The influence of glucagon on the secretion of enterokinase was first shown by Goldman et al

Enterokinase alkaline phosphatase and sucrase activities were measured in the duodenal mucosa of normal human adults before and after intravenous injection of 1 mg glucagon. A rapid and significant increase in mucosal enterokinase but not in alkaline phosphatase or sucrase was observed The same effect of glucagon was observed in a patient with complete obstruction of the biliary and pancreatic ducts Ultracentrifugation experiments showed that the transient increase of mucosal enterokinase activity was confined to the sediment but that the return to the basal activity was accompanied by a relative increase of soluble enterokinase in the mucosa. On intravenous administration of L-alanine endogenous plasma glucagons as well as mucosal enterokinase increased

Our findings suggest that hormones such as glucagon play an important role in the activity and release of muocosal enterokinase in man

A S McNeish & H Gaze (Birmingham) *The intestinal antibody response in infants with enteropathic E. coli gastroenteritis*

Serial samples of duodenal juice from a group of 12 infants with enteropathic E. coli (EEC) gastroenteritis were examined for specific anticoliform antibodies by bacterial agglutination. The specificity and cross-reactions of these antibodies were measured against a panel of EEC before and after absorption with a suspension of the specific infecting organisms. The classes of the agglutinating antibodies were determined by an indirect immunofluorescent technique with fluorescein isothiocyanate labelled antisera, and by absorption of the intestinal juice with specific antiimmunoglobulin antisera.

The antibody response in the duodenal secretions was detectable within 3 days of the infection, rose to a peak at about 14 days and returned to low levels with 25–30 days. Early in the response IgM antibodies were found, but later IgA antibodies predominated Antibodies at peak titre had greater specificity than early antibodies but cross-reactions were still demonstrable No significant serum antibody response was detected in these patients

These results are in accord with the findings of Porter in piglets immunized orally with antigenic extracts of E coli and also agree with the data of Girard & de Kalbermatten in infants similarly immunized

In both groups the bile acid concentration after the meal was often less than that required for the formation of micellar solutions and the solubilization of fat With human milk reasonable fat absorption occurred even with bile acid levels far below the critical micellar concentration In the babies fed with the cow s milk formula a striking association was found between the bile acid concentrations after the meal and the fat absorption coefficient

Compared with older infants and children premature babies excrete considerable amounts of bile acids in the faeces

E Guiraldes J E J Oyesiku S P Lamabadusuriya & J T Harries (London) *Selective inhibition of mucosal NA K ATPase by deoxycholate in rat jejunum in vivo*

Deoxycholate inhibits jejunal transport of glucose sodium and water and may play a role in the pathophysiological mechanisms operating in certain types of *infantile diarrhoea* (Harries & Sladen 1972) The mechanism by which this unconjugated dihydroxy bile salt inhibits transport however is *not* known *Active* transport of glucose is coupled to sodium transport and there is evidence that energy for this process is derived from hydrolysis of ATP by Sodium Potassium Adenosine Triphosphatase (NA K ATPase) We have investigated the simultaneous effects of Deoxycholate on *active* jejunal transport of glucose and sodium and on mucosal NA K ATPase

Both closed-loop and perfusion techniques were used in anaesthetised male Wister rats weighing 250–300 g. All solutions were isotonic (285 mOsm/kg) phosphate buffered to pH 7 4 and contained 2 or 20 mM glucose

In *closed-loops* 5mM deoxycholate selectively inhibited ($p<0\ 001$) mucosal NA K ATPase without affecting Mg ATPase

In the *perfusion studies* 5 mM and 1 mM deoxycholate inhibited NA K ATPase ($p<0\ 001$) At the lowest perfused concentration of deoxycholate (1 mM) there was a concomitant inhibition of *active* jejunal transport of glucose and sodium and of mucosal NA K ATPase activity

These results suggest that deoxycholate (1 mM) inhibits the *coupled* jejunal transport of glucose and sodium and water by inhibition of *mucosal NA K ATPase*

S Borulf & T Lindberg (Malmö) *Electrophoresis of human duodenal juice*

When analysis of duodenal juice is used for assessment of pancreatic function estimations of trypsin or amylase activity the two methods most generally used in clinical routine do not give any information about the whole spectrum of pancreatic enzymes Nor do they reveal the presence of inactive forms of these enzymes

Experiences with a simple and rapid method for simultaneous qualitative assay of different pancreatic enzymes (i e trypsin chymotrypsin elastase carboxypeptidase lipase and amylase) were reported

Duodenal juice collected before and after a test meal of water is submitted to electrophoresis in agarose gel Proteins are stained and enzymes identified by their action on specific chromogenic substrates

Differences in protein patterns and zymograms exist between normal individuals Specific patterns are obtained in different conditions of maldigestion At least two forms of trypsin are found in all juices running in different directions and of different stability

J Schmitz S W Bender V Troesch R Schneider & B Hadorn (Bern) *Subcellular localization of enteropeptidase (Enterokinase) in the human small intestinal mucosa*

Brush border bound disaccharidase activities are reduced in the atrophic mucosa of chil

digestive tract is now possible from both ends and digestive endoscopy has become a classical investigation tool in gastroenterology As the technique is harmless and well tolerated we were inclined to use it in children.

We used several Olympus fibroscopes all of them with a frontal view 4 directions of the distal end and a biopsy channel. Among them a paediatric mode (PCF=diameter 8 mm) proved very satisfactory allowing endoscopic examination of the upper and lower ends of the intestine even in the younger children General anaesthesia was usually needed except with the PCF where light sedation alone was sufficient.

In our present series 24 oesophagogastroscopies and even duodenoscopies in children aged 2 months to 13 years and 23 colonoscopies in children aged 1½ to 16 years have been performed. Indications and results were illustrated and discussed in comparison with radiological findings

J Perheentupa, C Holmberg & K. Launiala (Helsinki) *Congenital chloride diarrhoea I Clinical experience with 17 cases*

Maternal hydramnios always heralded the disease and most births were premature The length at birth was normal for gestational age whereas the weight tended to be increased reflecting intestinal accumulation of fluid. Passing of meconium has not been positively recorded in any case Instead a lot of fluid was excreted since the first day This had often been taken for urine but confirmation of its origin was easily done by introducing a catheter into the rectum a sample of liquid was usually obtained From 7 to 19% of weight was lost in the first day and hypochloremia and hyponatremia then tended to develop rapidly Some had rather high s-K and metabolic acidosis during the first weeks and only later a consistent tendency to hypokalaemia and alkalosis appeared The faecal chloride concentration decreased even to 40 mEq/l in the hypochloremic stage while its range was 110–180 mEq/l in the first days and during substitution Some had survived the neonatal period without adequate substitution a grossly abnormal steady state may obtain through cessation of diarrhoea in the state of hypochloremic dehydration which is associated with potassium deficiency alkalosis and degenerative kidney changes This praecarious balance may be lost through an infection. The objective of the treatment should be maintenance of completely normal water and electrolyte equilibrium by full replacement of the faecal losses The extra Cl requirement decreased slightly with age being for kg body weight and day 3.5–8 0 mEq during the first year and 2 0–7 0 mEq later One half to 3/4 of this should be given as NaCl and the rest as KCl We prefer using a fixed ratio of these salts in isotonic solution, and increasing the volume as required The most sensitive indicators of insufficient substitution are metabolic alkalosis, hypokalaemia and absence of Cl from urine During adequate therapy the pH and ion concentrations in ECF were normal as well as plasma volume and renin and angiotensin II activity The total exchangeable Cl^- and Cl space were larger than normal average by 5–10%

The angiotensin activity readily increased to high levels if the substitution was insufficient, but also if an excess of KCl was used in stead of the correct salt mixture This probably contributed to the arteriolar pathology reported earlier The proper treatment maintains the watery diarrhoea, but the children were able to fully control the bowel during the day at least by school age On an average they have 3 large bowel movements daily the growth is normal

C Holmberg K Launiala & J Perheentupa (Helsinki) *Congenital chloride diarrhoea*

R Lagercrantz P Perlmann S Hammarström & H E Karlsson (Stockholm) *Antibodies to colon in gastro-intestinal diseases*

Humoral antibodies to colon antigen were titrated by passive haemagglutination in sera from 500 patients with gastro intestinal disorders The following preliminary results will be discussed

1 The incidence of elevated titres was significantly higher in patients with ulcerative colitis Crohn s disease and hereditary colon polyposis than in healthy controls

2 Patients with gastroenteritis of viral bacterial or unknown origin had similar titres to those of the healthy controls

3 A group of patients with subacute or chronic gastro-intestinal disorders had titres intermediate between those observed in groups 1 and 2

4 The relatively small number of patients with recurrent urinary tract infections and serum viral hepatitis often had elevated antibody titres to colon

S Nordio M A Mangiarotti Marchi A G Marchi & G Mastella (Trieste) *Lymphoid cell beta glucuronidase and cystic fibrosis factor*

We pursued investigations on beta-glucuronidase activity of cultured lymphoid cells in cystic fibrosis (c f) Previous researches had shown that the activity of this enzyme is higher in both c f homozygotes and heterozygotes than in controls Nevertheless it shows no further increase on PHA stimulation either in homozygotes or in heterozygotes The response of the enzyme to PHA seems to be a marker for detecting c f heterozygotes

In thymidine incorporation and ultrastructure PHA-stimulated lymphoid cells of c f subjects do not differ from those of controls

Serum of both homozygotes and heterozygotes contains a factor (c f factor ?) which inhibits the beta-glucuronidase response to PHA of lymphoid cells from normal subjects Investigations show that this factor is resistant to heating at 56°C and to dialysis of sera from c f subjects

S Cadranel P Rodesch R Platteborse & H Loeb (Brussels) *Laparoscopy in children*

Direct visualization of intraperitoneal organs may be of considerable diagnostic interest and laparoscopy is a routine technique in adult gastroenterology With a paediatric model of the laparoscope now available it has become possible to perform this investigation in infants and children as well The technique is simple and especially in hepatic diseases very helpful liver biopsies can be taken under direct examination of the organ with greater safety and accuracy General anaesthesia is advisable to avoid undesirable movements of the patient In our service at the present moment 20 laparoscopies have been performed in children aged 3 months to 13 years on the following indications

(*a*) metabolic changes of the liver (mostly in infants)

(*b*) suspected portal hypertension as a late complication of catheterization of umbilical vessels in the neonatal period

(*c*) tumour of the liver

(*d*) prolonged or chronic hepatitis and cirrhosis of the liver (in older children)

These findings were illustrated and the results of our first experiences were presented In all cases laparoscopy was an interesting step in directing or achieving the diagnosis No complications were noticed

P Rodesch S Cadranel J P Peeters & M Cremer (Brussels) *Digestive endoscopy with fibre optics in children*

With the recent development of fibre optics in vivo examination of the mucosa of the

the response of the intestinal mucosa to the treatment

P. A. Krasilnikoff, H. Moltke & E. Gudmand-Høye (Hellerup): *The value of disaccharide tolerance tests in children*

A combined peroral lactose D-xylose tolerance test was made in 45 children 3 months to 10 years old. In 41 of the children a combined peroral sucrose D-xylose tolerance test was made as well. Each child received ? g per kg of disaccharide and 15 g per m^2 of D-xylose.

During the lactose test a *maximal* blood sugar rise of less than 20 mg per 100 ml was found in 14 cases (31%) and a *maximal* rise of less than 25 mg per 100 ml in 21 cases (47%). Nineteen of the children with the lowest rise in blood sugar were further given lactose D-xylose directly into the duodenum through a tube. After this all but three children suffering from coeliac disease and one child suffering from lactose malabsorption showed a perfectly normal rise in blood sugar. The lactase activity in a jejunal mucosal biopsy was related to the rise in blood sugar. The lactase activity in a jejunal mucosal biopsy was related to the rise in blood sugar in 20 cases. In six of these a tose test and the lactase activity but in all cases conformity with the blood sugar rise after duodenal instillation was good.

Exactly the same results were obtained during the tolerance tests with sucrose D-xylose.

There was no difference in the rise of D-xylose in the blood during the peroral and the duodenal tolerance tests. Slow emptying of the stomach is thus not likely to be the reason for the great number of a false-positive peroral lactose and sucrose tolerance tests. Other possible reasons are discussed.

S. P. Lamabadusuriya & J. T. Harries (London): *The influence of micellar solutions on the inhibitory effects of deoxycholate on jejunal transport*

Deoxycholate, an unconjugated dihydroxy bile salt, is produced by bacterial degradation of primary trihydroxy bile salts and occurs in the small intestinal lumen of man in conditions of bacterial overgrowth; it inhibits fluid and glucose absorption and may play a role in the pathophysiology of certain types of diarrhoea.

We have investigated the influence of micellar solutions on the effects of deoxycholate on fluid and glucose transport in rat jejunum using an *in vivo* closed loop technique. Both 2.5 and 5 mM deoxycholate produced secretion of water; 5 mM deoxycholate inhibited glucose absorption ($p < 0.001$). These effects persisted when deoxycholate was solubilised in pure bile salt micelles (using 15 and 25 mM Taurocholate).

Mixed Taurocholate (15-20 mM) micelles containing 0.25, 0.75, 2.5 and 10 mM Oleic acid were prepared, and 2.5 mM deoxycholate was soluvilised in each of the solutions. With increasing concentrations of Oleic acid there was a concomitant reduction in the secretory effects of deoxycholate and at 2.5 and 10 mM net absorption occurred. Caprylic acid and Monolein mixed micelles showed a similar effect. Oleic acid (10 mM) emulsions had no effect on the secretory effects of deoxycholate.

The inhibitory effects of 5 mM deoxycholate on glucose absorption were abolished by mixed micellar solutions (20 mM Taurocholate, 10 mM Oleic acid).

The Rate of Disappearance of ^{14}C Deoxycholate from the jejunal loops was reduced ($p<0.01$) by soluvilisation in *mixed* micellar solutions but not by solubilisation in *pure* bile salt micelles.

These results show that mixed micellar solutions protect against the inhibitory effects of deoxycholate on jejunal fluid and glucose transport and that protection may be achieved by solubilisation of deoxycholate within the expanded hydrocarbon core of the mixed micelle. The clinical effects of deoxycholate in man may thus be dependent

II Studies on the colonic absorption of electrolytes

Colonic perfusion studies were performed in three CCD patients and three healthy siblings A double lumen tube was brought into the caecum and ^{36}Cl was added to the perfusion solution for measurement of Cl^- influx and efflux

1) In perfusion of the normal colon with a physiological solution net absorption of Na^+ water and Cl^- and slight secretion of K^+ were observed In CCD absorption of Na^+ and water were decreased K^+ was secreted at a higher rate and both influx and efflux of Cl^- were almost zero

2) When sulfate was substituted for HCO_3^- in the controls absorption of Na^+ and water decreased HCO_3^- was secreted and Cl^- efflux was clearly reduced CCD patients showed practically no absorption of Na^+ and water Cl movements were almost absent and no HCO_3^- was secreted

3) Reduction of the Cl^- concentration stepwise to 60 and 0 mM led to a decrease in absorption of Na^+ water and Cl^- in controls whereas in CCD there was no such change

A new model for the normal colon was proposed with Cl^- and HCO_3^- moving in both directions by means of a Cl^-/HCO_3^- exchange This theory is supported by 1) decreased Cl^- efflux in absence of luminal HCO_3^- 2) impairment of both influx and efflux in CCD and 3) impairment of HCO_3^- efflux in CCD

The basic defect of CCD i failure of Cl^-/HCO_3^- exchange in the ileum and colon

F Carswell & R Lindsay (Bristol) *Erythrocyte sodium and potassium concentrations in coeliac disease*

Increased sodium loss into the bowel lumen has been reported in both treated and untreated coeliac patients This could arise because of increased sodium/potassium ATPase activity in the enterocytes Accordingly we have examined the concentrations of sodium and potassium and the sodium/potassium ATPase activity of the R.B C s of patients with coeliac disease

In 9 patients with untreated coeliac disease the R B C s contained a lower concentration of sodium and a higher concentration of potassium and there was significantly more ATPase activity in the R B C.s than in 12 control patients In 20 coeliac patients who had been on a glutenfree diet there were no significant differences from these control values Coeliac patients who had been on the gluten-free diet for less than a year had significantly higher sodium/potassium ATPase activity than patients who had been on the diet for more than 1 year

We are currently testing the hypothesis that coeliac plasma contains a factor which increases the sodium/potassium ATPase activity of the R B C s

J Jodl Z. Lojda & J Štěpán (Prague) *Isoenzymes of alkaline phosphatase in serum of children with coeliac sprue*

Histochemical examination of the intestinal mucosa in patients with coeliac sprue shows that the activity of the brush border alkaline phosphatase of enterocytes which is selectively inhibited by L-phenylalanine decreased in dependence on the stage of the disease The L phenylalanine sensitive fraction of the alkaline phosphatase in the blood serum is considered to be of intestinal origin The authors studied the behaviour of the serum alk phosph and its L phenylalanine sensitive fraction in patients with coeliac sprue and with other forms of the malabsorption syndrome In patients with coeliac sprue in the acute stage a significant increase of the L phenylalanine sensitive fraction was observed After a gluten free diet a decrease of this fraction was seen within 3–5 days after the beginning of the diet. In patients with coeliac sprue this procedure promises to be a simple method for indirect assessment of

the response of the intestinal mucosa to the treatment

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on its physiochemical state in the intestinal lumen

Th Lücking & R Grüttner (Hamburg) *Rat intestinal enzyme activities during oral neomycin and gliadin administration*

For several years it has been known that oral neomycin administration causes a reversible malabsorption syndrome in man It was further found that neomycin induced malabsorption could be prevented by application of a gluten free diet Therefore a relationship to gluten sensitive coeliac disease with regard to pathogenetic mechanisms has been considered After successful demonstration of neomycin-induced malabsorption in the rat as well it appeared possible that a gluten-dependent enteropathy could be produced experimentally

We investigated the effect of neomycin and the role of dietary gliadin on several rat intestinal enzyme activities (3 disaccharidases 2 dipeptidases 2 phosphatases and β-glucuronidase) and on the morphology of the mucosa No differences were detected between the animal groups receiving neomycin and a gliadin-free or a gliadin-containing diet and the control groups

Our results are in contrast to the findings of Peternel et al but in accord with the data of Freier et al We are in agreement with other authors who also found no intestinal changes after neomycin treatment that there is no necessity for further studies on this subject

K H Niessen P Osswald K Schmidt G Brügmann F Hartmann & E Droste (Tübingen) *Studies on the pathophysiological significance of secondary enterokinase deficiency of the duodenal mucosa*

Total as well as partial atrophy generally leads to enterokinase deficiency of the duodenal mucosa To clarify whether this finding is of pathophysiological significance for protein digestion we measured enterokinase activity in homogenized mucosa specimens of 35 children Trypsin and chymotrypsin activities were determined in the duodenal juice after stimulation of the pancreas with secretin and pancreozymin On the basis of a very significant difference in enterokinase activity patients were divided into two groups of equal size regardless of histological findings in the mucosa Group 2 showed an enterokinase activity 58% lower than group 1 ($p<0\ 0005$)

Comparison of the activities of trypsin and chymotrypsin in the third 10-minute phase after stimulation with secretin and the first 10-minute phase after stimulation with pancreozymin showed no difference between the two groups either in activities per kg body weight or in concentrations Equally there were no significant differences in enzyme activities between the 30 minute secretin phases ($p<0\ 10$) or the 30-minute pancreozymin phases ($p<0\ 30$) although the enterokinase activities were highly significantly different

These in vivo investigations show that secondary enterokinase deficiency of the duodenal mucosa in contrast to other enzyme defects probably has no clinical significance

E Rossipal (Graz) *On the incidence of coeliac disease in Styria Austria*

The frequency of coeliac disease in the south-eastern part of Austria in infants and children not more than two years of age was estimated by the number of cases seen in a period of twelve months This number was related to the total number of children in the corresponding age group living in the area served by our clinic From this relation the incidence of coeliac disease in infants and young children was estimated to be 1 in 550 Marker diseases like pyloric stenosis and cystic fibrosis were also used for estimating the incidence of coeliac disease

Margot Shiner Aileen O B Redmond & J D L. Hansen (London): *The jejunal mucosa in protein-energy malnutrition before and after treatment*

This study attempts to throw light on the fine structural appearances of the jejunal mucosa in children with protein-energy malnutrition (PEM) in relation to the severity of the disease and response to therapy Five children with PEM were studied before and several days after treatment. The 3 most severely ill patients had a villous and glandular atrophy Ultrastructurally the absorptive cells were grossly disorganized and showed a decrease in endoplasmic reticulum, dilated vesicles and numerous vacuoles probably of phagocytic type The fine structure of the connective tissue the endothelium and the inflammatory cells was also abnormal In the 2 less severely ill patients intermediate changes were seen It was concluded that the grossly altered mucosa in PEM responds poorly to oral feeding and that this should be suspended in favour of intravenous alimentation

J Salazar de Sousa & J Pascoal Duarte (Lisbon): *Fingerprints in childhood coeliac disease*

Nine children aged 2 to 15 years with previously diagnosed coeliac disease were submitted to intestinal biopsy and their finger prints were taken None of them was following a gluten-free diet.

The intestinal mucosa in all had a flat appearance under the dissecting microscope and subtotal villous atrophy was shown by the optical microscope No abnormalities were found in their fingerprints as compared with normal controls of the same age

We conclude that fingerprints are of no diagnostic help in childhood coeliac disease in contrast with what has been claimed in [illegible]

J A Walker-Smith B Turner & J Blomfield (London) *Therapeutic implications of copper deficiency in Menkes kinky hair syndrome*

Copper deficiency in children with Menkes kinky-hair syndrome was first reported by Danks et al. in 1972 In two children studies of copper absorption revealed evidence of malabsorption of this metal

Parenteral therapy with copper EDTA in an infant with copper deficiency in Menkes kinky-hair syndrome is described This infant had radio-isotopic evidence of copper malabsorption and had failed to respond to oral copper Intramuscular copper EDTA given in daily injections containing 1 mg copper raised his total plasma copper and copper oxidase reflecting caeruloplasmin to normal levels The liver copper level prior to therapy was 20 1 μg/g and this rose to 34 μ/g following therapy

There was no neurological improvement however and the infant died from bronchopneumonia.

It is probable that infants with Menkes kinky-hair syndrome have adequate copper stores at birth as clinical manifestations do not appear for some weeks when they have presumably become copper-depleted owing to malabsorption of copper Early diagnosis in infants born into families at risk, by serial plasma copper estimations before clinical manifestations have developed appears to offer the best chance for possible successful management with parenteral copper Once central nervous system damage has occurred relief of the state of copper deficiency is unlikely to produce any therapeutic benefit.

Long-term observations of infants with this syndrome treated with parenteral copper EDTA will reveal the value of this form of therapy and further investigation is necessary to clarify the nature of the copper malabsorption found in this disorder

Pekka Kuitunen

NEW BOOKS RECEIVED

M Bessis *Living blood cells and their ultrastructure* 767 pp illus Springer Verlag Berlin Heidelberg and New York 1973 DM 151 —

G Gladtke & H M von Hattingberg. *Pharmakokinetik* 148 pp illus Springer Verlag Berlin Heidelberg and New York 1973 DM 19 80

M I Griffiths (ed) *The young retarded child Medical aspects of care* 227 pp Churchill Livingstone Edinburgh and London 1973 £3 00

M A Lennox Buchthal. *Febrile convulsions A reappraisal* 138 pp illus Elsevier Scientific Publishing Company Amsterdam London and New York 1973 No price given

J W A Meijer H Meinardi C Gardner Thorpe & E van der Kleijn (eds.) *Methods of analysis of antiepileptic drugs* 257 pp illus Proceedings of the Workshop on the Determination of Anti-eleptic Drugs in Body Fluids Noordwijkerhout The Netherlands 13–14 April 1972 Excerpta Medica Amsterdam American Elsevier Company Inc New York 1973 D Fl 69 00

F Schmid (ed) *Pädiatrische Radiologie* Band I Stützgewebe Zentralnervensystem Syndrome 580 pp illus DM 248 — Band Thoraxorgane Verdauungstrakt Urogenitaltrakt 525 pp illus DM 248 — Springer Verlag Berlin Heidelberg and New York 1973

R L Wesenberg *The newborn chest* 300 pp illus. Harper & Row Publishers Hagerstown Maryland New York Evanston San Francisco and London 1973 US $20 00

H L. Barnett & A H Einhorn (eds) *Pediatrics* 15th ed 2070 pp illus Appleton-Century-Crofts Educational Division Meredith Corporation New York 1973 $29 15

G H Bourne (ed) *World review nutrition and dietetics* Vol 17 318 pp illus. S Karger Basel München, Paris and London 1973 £20 90

G Galli G Jacini & A Pecile (eds.) *Dietary lipids and postnatal development* 278 pp illus Raven Press Publishers New York 1973 D Fl 50 00

U S Department of Health Education and Welfare Public Health Service Vital and Health Statistics Series 20 No 14 Rockville Maryland 1973 *A study of infant mortality from linked records by age of mother total-birth order and other variables* 55 pp DHEW Publication No (HRA) 74–1851 85 cents

J Apley *Paediatrics* 441 pp Illus Ballière Tindall London 1973 £2.50

Family planning in the education of nurses and midwives Public Health Papers No 53 World Health Organization Geneva 1973 65 pp

C E Field & F M Baber *Growing up in Hong Kong A preliminary report on a study of the growth development and rearing of Chinese children in Hong Kong* 178 pp illus Hong Kong University Press, Hong Kong 1973 HK $35 00

S S Gellis & B M Kagan (eds) *Current pediatric therapy-6* 870 pp W B Saunders Company Philadelphia 1973 £11 30

D F Horrobin *Prolactin physiology and clinical significance* 239 pp Medical and Technical Publishing Co Ltd, Lancaster 1973 No price given

K Kanig *Einführung in die allgemeine und klinische Neurochemie* 17 pp illus Gustav Fischer Verlag. Stuttgart 1973 DM 1 .80

J S Payne R A Payne C D Mercer & R G Davison *Head start A tragicomedy with epilogue* 253 pp illus Behavioral Publications New York 1973 $4 95

A Ravic *Preventing V D and cancer by circumcision* 211 pp Philosophical Library New York 1973 $9 95

B Robertson *Micro-angiography of the lung in infancy and childhood* 86 pp illus Proprius Stockholm 1973 No price given

G Seifert (ed) *Verhandlungen der Deutschen Gesellschaft für Pathologie 56 Tagung vom 16 bis 20 Mai 1972 in Graz* 749 pp illus Gustav Fischer Verlag. Stuttgart 1973 DM 4 —

S H W Sell & D T Karzon (eds.) *Hemophilus influenzae* 325 pp illus Proceedings of a conference on antigen-antibody systems epidemiology and immunoprophylaxis April 4–5 1972 Vanderbilt University Medical School Nashville Tennessee Vanderbilt University Press, Nashville 1973 $15 00

L C Taichert *Childhood learning behavior and the family* 112 pp Behavioral Publications, New York 1973 $7 95

U S Vital and Health Statistics Data from the National Health Survey Serie 11 No 1 4 *Height and weight of youths 12–17 years United States (1966–70).* 81 pp DHEW Publication No (HSM) 73-1606 $1 00

BOOK REVIEWS

A. J. Raimondi: *Pediatric neuroradiology* 716 pp Illus. W. B. Saunders Company Ltd., Philadelphia, London, Toronto 1972. £19 15

The increasing importance of neuroradiology in pediatric diagnosis has emphasized the need of a comprehensive treatise in this highly specialized field. The monograph briefly reviewed here reflects the author's personal experience with more than 4 000 neonates, infants, and children and should be welcome to all those concerned with pediatric aspects of neuroradiology.

Far from being elementary the book contains no description of the radiologic procedures employed but meticulously accounts for the radiologic recognition and localisation of abnormalities occurring in the main varieties of intracranial diseases and malformations. A large number of illustrative radiographs and colour drawings help to integrate the information afforded by pneumoencephalography and cerebral angiography. The diagnosis of vertebral and medullary spinal lesions has been omitted but a short chapter on the radiology of the skull—written by H. White—is included.

G. Theander

J. L. Melnick (ed.): *Progress in medical virology* vol. 14. S. Karger, Basel, München, Paris, London, New York, Sydney 1972. 339 pp. Illus. US $26.60

The 1972 volume of the series of Progress in Medical Virology comprises eight chapters on items of cardinal interest. Peter Dent gives an informative review of the state of knowledge concerning the problem of immunodepression by oncogenic viruses. In all model systems with these viruses defects in antibody production have been found, while data on cellular immunity are equivocal. Dent suggests that the mechanism of immunodepression may differ in short and in long-incubation systems. He also tries to evaluate the role of immunodepression in the malignant process: is it a necessary antecedent or a consequence of oncogenesis? The chapter comprises a very instructive summary of the current concepts of humoral and cellular immunity.

Until recently viruses were generally considered to contain a single molecule of nucleic acid. In recent years evidence has indicated that more than one genome is regularly contained in a number of virus groups and the term multiploid has been introduced. Edward Simon reviews and discusses the present state of knowledge in this field. Y. Z. Ghendon gives a comprehensive review on methods of obtaining conditional lethal mutants of animal viruses, on their analysis and on the results of studies on functional defects in mutants of several viruses. He illustrates different ways of obtaining information on the function of virus genes and the replication of viruses by means of investigations of conditional lethal mutants. He concludes that such work may also prove to be useful for practical virologic problems such as selection of criteria to be used for choosing vaccine virus strains. Dana Sergescu, Florian Horodniceanu and Andrei Aubert-Combiescu survey and evaluate the use of inhibitors in the study of picornavirus genetics.

To every one working in the field of infectious diseases the informative survey by Jean Joncas on the clinical significance of EB herpes virus infection in man is of great interest. Viewpoints range from EB virus being a direct cause of mononucleosis to the concept of a harmless passenger virus. He presents a hypothesis of the disease being an early or late postinfectious syndrome. An interesting aspect of the presentation is the concept of multicausal disease.

The article by G. D. Hsiung dealing with the various strains belonging to the para-influenza-5 group of viruses is of particular interest to people working in diagnostic and research virology laboratories. The possibility that viruses isolated from clinical specimens are adventitious para-influenza-5 viruses latently infecting monkey kidney cell cultures is a considerable practical problem. The valuable chapter by Blaškovič and Nosek deals with a tropic of great clinical interest—the ecological approach to the study of tick-borne encephalitis. The authors illustrate the contributions that an ecological approach can make to an understanding of the epidemiology and control of the disease. Based on their own extensive experience from the Tribeč area of Czechoslovakia, recommendations are made for surveillance and for application of public health measures to protect human population groups at risk.

In the final chapter of the volume & the Editor gives his customary distinguished report on progress in classification and nomenclature of animal viruses. In the present chapter current status of the work of the International Committee on Nomenclature of Viruses—as published in 1971—has been taken into account and certain new findings have not yet officially been approved.

Gun Carlström

H Begemann & J Rastetter: *Atlas der klinischen Hämatologie* 2nd ed Springer Verlag Berlin Heidelberg New York 1972, 374 pp illus DM 248.–

This book is the second edition of the well-known atlas by Heilmeyer and Begemann It contains mainly illustrations with texts An account of the puncture technique of the bone marrow the lymph nodes and the spleen is given as an introduction and it is followed by a chapter on haematological staining technique including some widely used cytochemical methods The normal cytology of bone marrow spleen and lymph nodes is presented in detail and the main part of the book is devoted to diseases of these organs There is also a short chapter on electron microscopy of bone marrow and blood cells and on the diagnosis of parasitic disease

The typography is excellent and the book contains abundant drawings and colour photographs A systematic review is given of the most common haematological disorders with texts and pictures well arranged on the same openings. The index is adequate

The book seems to be a compromise between a purely pictorial presentation and a short textbook on haematology It may be questioned whether a detailed description of puncture technique belongs to an atlas of this character Furthermore the short chapter on electron microscopy seems superfluous as many special works are available on this subject

Owing to its high price the book has no place in pregraduate teaching or as an everyday laboratory manual It is however valuable for anyone interested in general haematology but gives no special information on paediatric haematology

Alf Rausing
Hans Ekelund

E Gautier & L. S Prod'hom (eds): *Gehörstörungen beim Kind* Pädiatrische Fortbildungskurse für die Praxis, Vol 34 S Karger Basel 1972 99 pp sFr 33.–

This book is a new volume in the postgraduate series "Pädiatrische Fortbildungskurse für die Praxis" The topic is covered in eight chapters contributed by different authors Graf gives a good introduction with a review on etiology and pathogenesis. Then comes a paper by Mathis presenting symptoms and diagnostic tests used by the author It is rather astonishing that nothing is mentioned about objective conditioned-reflex audiometry which is very valuable in the age group 1–3 years when used with the peep-show technique

Two papers are dealing with ERA (Evoked Response Audiometry) which is an objective way of registering cortical responses to auditory stimuli Nowadays there is a great interest in this field all over the world and a thorough review of the method is given However the method has still a restricted value in clinical practice.

Interesting facts regarding psychological problems and changes in perception in the hard of hearing and deaf children are presented in two papers by Affolter and Ajuriaguerra Finally teaching and writing problems in this group of children are discussed As a whole a very interesting volume but one must regret that a chapter on therapy was not added especially with regard to ear surgery and hearing aids

Sten Harris

ANNOUNCEMENT

The Fifth Congress of the Union of European Pedopsychiatrists is to be held in Vienna Austria from June 30 to July 5 1975

For information and registration please apply to D F Poustka, Scientific Secretary c/o Wiener Medizinische Akademie Alserstrasse 4 1090 Wien Österreich.

Acta Paediatr Scand 63: 673–678, 1974

MALIGNANT LYMPHOMAS IN CHILDREN
A CLINICO-PATHOLOGIC RETROSPECTIVE STUDY

I. *Hodgkin's Disease*

S. GARWICZ, T. LANDBERG and M. ÅKERMAN

From the Departments of Paediatrics, Radiotherapy, Cytodiagnostics and Pathology, University Hospital, Lund, Sweden

ABSTRACT. Garwicz, S., Landberg, T. and Åkerman, M. (Departments of Paediatrics, Radiotherapy, Cytodiagnostics and Pathology, University Hospital, Lund, Sweden). Malignant lymphomas in children—A clinico-pathologic retrospective study. I. Hodgkin's Disease. Acta Paediatr Scand, 63: 673, 1974.—Data on 23 children with Hodgkin's disease from a 29-year period are given. Sixteen were boys and 7 girls. At microscopic review of the biopsy material the lesions were typed according to Lukes. Three had Lymphocyte predominance, 7 had Nodular sclerosing type, and 13 had Mixed cellularity. Viewed with the limited staging procedures that often had been used, 17 patients had at presentation localized disease (stages I or II), 4 had generalized lymphadenopathy and 2 had extralymphatic disease. Treatment had been given with radiation therapy or chemotherapy or the two combined. Data on local recurrence after radiotherapy is in line with other reports, indicating that Hodgkin's disease in children responds in the same way to irradiation as in adults. Fifteen of the 23 are still alive mean 69 months after the initial treatment, 6 of them having been followed for at least 8 years. Of 13 patients evaluable for 5-year survival 6 were alive at 5 years. Children with Hodgkin's disease appear to have similar distribution on histologic types, progression of disease and response to treatment as adults. It seems that similar clinical evaluation procedures and similar therapeutic techniques should be used in Hodgkin's disease in children as in adults.

KEY WORD: Hodgkin's disease

Malignant lymphomas constitute, next to leukemia and tumours of the nervous system, the largest group of malignant disease in children in Sweden, and during a 7-year period from 1959 to 1965 (2) there were totally 129 new cases of malignant lymphomas among children of at most 14 years of age. Of these, 43 were diagnosed as Hodgkin's disease, 28 as reticulum cell sarcoma, 26 as lymphosarcoma, 19 as malignant lymphoma NUD and 13 as Brill-Symmer's disease or reticulosis.

During the 29-year period 1944 to 1972 a total of 50 children with malignant lymphomas were first seen at the Departments of Paediatrics and of Radiotherapy in Lund. At review, 23 of the children were found to have Hodgkin's disease, whereas the remaining 27 showed non-Hodgkin's lymphomas. The purpose of the present investigation is to report on the 23 children with Hodgkin's disease. Extrapolation of the figures given by the Swedish Cancer Registry (2) indicate that during the years 1944–72, there may have been about 27 new cases of Hodgkin's disease in children in the region served by the University Hospital of Lund, and the present series of pa-

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Nodular sclerosing type Similarly Chaves (3) found only one out of 14 children in the first decade to have the Nodular sclerosing type, and in his material Mixed cellularity was the most common type and accounted for 9 of the patients

Young et al (17) in a study of mainly older children found 9 to have Lymphocyte predominance 15 to have the Nodular sclerosing type 10 to have Mixed cellularity and 4 to have Lymphocyte depletion

Clinical Data

During the early period covered by the present series only limited staging procedures were carried out, and thus only 7 patients had a urography 6 a lymphography and 3 (XIII D J XIV K P and XV C A) a staging laparotomy with splenectomy

Ten of the 23 patients had at presentation stage I disease (Table 1) 7 had stage II 4 had stage III and 2 had stage IV Three of the patients (XV C A XXI M K Å and XXII J P A) had constitutional symptoms A similar distribution on stages appears in the series of Strum & Rappaport (15) 7 of their patients having stage I 5 stage II 4 stage III and 1 stage IV But Pitcock et al (12) reported 29 of their patients to have localized disease whereas 15 had generalized disease and in the series of Jenkin et al (7) 32 had stage I or II disease whereas 22 had stage III

In the present series 11 patients had at presentation mediastinal involvement One of them had Lymphocyte predominance 4 had the Nodular sclerosing type and 6 had Mixed cellularity In the series of Strum & Rappaport (15) almost all children with mediastinal presentation had Nodular sclerosing type

Four of the patients had a hemoglobin value below 70% (=11 2 g/100 ml). Leucocytosis was common but only 2 patients had a leucopenia of less than 4000/mm³ and these 2 patients (II F J and XII N S) also had lymphopenia of less than 1000/mm³ whereas none of the other patients had a lymphopenia The ESR was usually raised only 3 patients having at most 10 mm/l hr and 14 had at least 25 mm/l hr

Twenty-one of the 23 patients (Table 1) had at presentation only involvement of lymph nodes or spleen In 10 more than one lymph node group was involved Of these 10 7 had only lymph node involvement and in all 7 were all involved lymph node groups adjacent Three had in addition to contiguous lymph node group involvement also involvement of the spleen and in one of them the mesenteric lymph nodes were found at laparotomy to be involved

Extensions were seen in 8 of the 21 patients who at presentation had only involvement of the lymph node groups or spleen (Table 1) In 4 the first extensions occurred in only adjacent lymph node groups in one patient who had cervical lymphadenopathy the first extension occurred in abdominal lymph nodes and in the spleen and in the remaining 3 patients the first extensions were extralymphatic

Thus also in paediatric Hodgkin s disease the pattern of spread of Hodgkin s disease seems to be largely predictable and thus also appears in the series of Jenkin et al (7)

At the end of the follow-up splenic involvement had been demonstrated in 9 patients involvement of the liver in 7 and of the lung tissue in 3 None of the patients showed leukemic transformation

Treatment

The initial treatment (Table 1) consisted of involved field irradiation in 12 patients of extended field irradiation in 8 (in one of them in combination with single drug chemotherapy) and of chemotherapy in 3

It does not seem to be settled which radiation absorbed dose that should be given to eradicate Hodgkin s disease in

tients may therefore be relatively representative of Hodgkin s disease in children in the population The series with non Hodgkin s lymphomas will be reported later (5)

Hodgkin s disease in children has been known for 142 years The report by Thomas Hodgkin on 7 cases in 1832 (6) included 2 children namely Case I Joseph Sinnott aged 9 and Case II Ellenborough King aged 10 Also in the history of the treatment of Hodgkin s disease paediatrics has pioneered since the first patient reported to have been treated with radiation therapy for Hodgkin s disease was a child of 4 (13)

Sex and Age

The sex and age for each patient is given in Table 1 Sixteen were boys and 7 were girls and a sex ratio of the same order has been reported by other authors (1 7 12) Fraumeni & Li (4) found a similar sex quotient up to the age of 10–12 years but above that age the quotient tended to become smaller Young et al (17) however reported 28 males and 10 females in a series with a mean age of 13 and a range of 6–16 only 6 out of the 38 being below 10 years of age However a marked male predominance of the order of 90% in Hodgkin s disease in the first decade has been reported by Uddströmer (16) Strum & Rappaport (15) and Schnitzer et al (14) and also appears in the present series where 9 out of 11 patients in the first decade were boys

The disease has only rarely been reported below the age of 4 Pitcock et al (12) reported 3 such patients Bailey et al (1) one and Jenkin et al (7) 3

Microscopy

Biopsy or post mortem tissue was obtained in all cases and from 1964 on also cytologic material (fine needle biopsies or touch preparations) in some cases All material was reviewed in detail by one of the authors (M Å) and categorized on the basis of recent concepts in histopathological classification

Hodgkin s disease was subdivided according to Lukes (10) into four categories Lymphocyte predominance Nodular sclerosing type Mixed cellularity and Lymphocyte depletion

The histologic type in the initial biopsy is given for each patient in Table 1 Three of the patients had Lymphocyte predominance 7 had Nodular sclerosing type and 13 had Mixed cellularity It may be noted that 6 patients had a history of at least one year 2 of them having Lymphocyte predominance and 4 having Mixed cellularity Remarkably 11 of 13 patients with Mixed cellularity were boys

In reports on Hodgkin s disease in children usually the classification of Jackson & Parker has been used Using this classification Pitcock et al (12) however with reference to Lukes & Butler (9) remarked that they had not observed the nodular form of sclerosis in their series

In a study of Hodgkin s disease in the first decade Strum & Rappaport (15) found 30 children out of 31 to have either Lymphocyte predominance or the Nodular sclerosing type of disease In the present series 2 of the 11 patients of at most 10 years of age had Lymphocyte predominance 2 had the Nodular sclerosing type and 7 had Mixed cellularity The difference between the materials may be due to selection factors but there may also be an epidemiologic difference since in a material of 149 mainly adult patients with Hodgkin s disease from the same region as the present series (Landberg & Larsson 8) Mixed cellularity also was the most frequent type accounting for 54% of the patients whereas only 21% had the Nodular sclerosing type and 12% Lymphocyte predominance Schnitzer et al (14) found the Nodular sclerosing type to be the most frequent accounting for 27 children out of 55 but of the 21 patients in their series in the first decade only 7 had the

treatments that had been given with conventional orthovoltage roentgen rays proved only in some instances to be evaluable retrospectively with reasonable certainty as regards distribution of the absorbed dose in the target. Therefore the study was limited to include 6 patients who had presented with mediastinal disease and who had been treated with cobalt-60 radiation therapy In 4 of them (VIII J M XII N S XIII D J and XVIII L. J Å) having been followed up for 49–17 months no signs of a local recurrence appeared The absorbed dose in the target in these 4 patients had been between 3500 and 4100 rad given in 21–27 fractions over 61–83 days split-course treatment In one of the patients (XI R. E) a recurrence in the sternum was diagnosed 23 months after the beginning of mantle treatment at the same time there was a generalisation of the disease In the last of the 6 patients studied (XVII J B) a recurrence in the mediastinum appeared as the first new sign of disease This patient had received an absorbed dose in the mediastinum of 2200 rad given in 14 fractions in 19 days, and the total absorbed dose for this patient was thus below the one given to the recurrence-free patients Also when judged with the concepts of NSD and TSD this patient had received smaller doses (NSD= 850 ret, and TSD=1200 ret, respectively) compared with the other 5 patients in whom the corresponding values were at least 1000 and 1600 ret respectively and in 3 of the 5 above 1100 and 1900 ret respectively The data is anecdotal and suffers from too short an observation time but is in line with the opinion expressed by Paterson (11) that in the radiotherapy of Hodgkin s disease in children the same absorbed dose should be given as in adults Paterson suggested that 3000 rad in 3 weeks should be given in localized forms of the disease and Jenkin et al. (7) found recurrence to be relatively common after less than 2500 rad and they recommended 3500 rad Young et al (17) used the same treatment techniques and tumour absorbed doses (3500–4000 rad) as in adult patients and they recommended split-course treatment over 6–8 weeks.

In 3 of the patients in the present series chemotherapy was given as *sole initial treatment*. One of them (XV C A) had generalized lymph node involvement including mesenteric lymphadenopathy received combination chemotherapy and was in remission 7 months later at the end of the follow-up Two patients (XXII J P A and XXIII C B) received single-drug chemotherapy because of advanced disease and poor general condition respectively the survival for these 2 patients being only 3 and 1 month, respectively

In a study of 38 children with Hodgkin s disease Young et al (17) found that the results of intensive radiotherapy or combination chemotherapy were significantly superior to published reports of less intensive radiotherapy or single drug chemotherapy

Five patients in the present series later received treatment with both radiation therapy and single drug chemotherapy 2 received later only radiation therapy and one only chemotherapy

Survival

Fifteen of the 23 patients are still alive 195–7 (median 49 mean 69) months after the beginning of treatment Three of them have had extensions whereas the others have been symptom-free after the initial treatment

Eight patients have died 56–1 (median 15 mean 24) months after the beginning of treatment all in advanced Hodgkin s disease.

Of the survivors 6 have a follow-up of at least 5 years all 6 in fact having been followed for at least 8 years Four of them have stayed symptom-free since the initial treatment, and they may stand a chance of

Table 1 *Sex age clinical data histologic type in initial biopsy Initial treatment and follow-up data for 23 children with Hodgkin s disease*

M=Male F=Female L.p =Lymphocyte predominance N s.=Nodular sclerosing type M c =Mixed cellularity ●=manifestations at first treatment ○=later manifestations Mw=bone marrow 0=skeleton Me=mesenteric lymph nodes E=epipharynx e=epidural L=lung IF=involved-field radiation therapy EF=extended-field radiation therapy TNI=total nodal irradiation SDCT=single drug chemotherapy MOPP=combination chemotherapy a=alive d=dead

					Clinical localizations of disease												
Patient	Sex	Age	Length of history (mos)	Histol type in biopsy	Neck right	Neck left	Axilla right	Axilla left	Mediastinum	Retroperitoneal	Groin right	Groin left	Spleen	Liver	Others	Initial treatment	Follow-up (mos.) after first treatment and status at end of follow-up
I H T	M	15	2	N s					●							IF	195 a
II E J	M	14	4	M c		●			●							IF	147 a
III G A	M	13	12	M c	●	○	○	○	○	○	○	○		○	○Mw	IF	112 a
IV E M L.	F	14	3	N s		●										IF	108 a
V G B A	F	15	5	N s	○	●				○			○			IF	104 a
VI L. B	F	10	17	L p	●											IF	103 a
VII A P	M	7	19	M c	●	●	●	●								EF	49 a
VIII J M	F	11	1	M c	●				●							EF	49 a
IX N K	M	13	1	M c		●			●				●			EF+SDCT	48 a
X M J	M	8	33	L.p		●										EF	35 a
XI R E	F	15	5	L p		●		○	●					○	○0	EF	79 a
XII N S	M	6	6	M c	●											EF	29 a
XIII D J	M	14	3	M c	●	●		●	●				●			TNI	17 a
XIV K P	M	6	5	N s		●										EF	11 a
XV C A	F	15	6	N s	●	●	●	●	●	●			●		●Me	MOPP	7 a
XVI H P	M	4	16	M c	●	○			○				○	○	○E+e	IF	56 d
XVII J B	M	14	2	N s	●	●		○	●	○				○	○L	IF	51 d
XVIII L J Å	M	10	3	N s	●	○	○	○	●				○		○L	IF	43 d
XIX K S	M	9	12	M c	●	○	○		●		○	○				IF	22 d
XX J L	F	7	8	M c	○	●			○				○	○		IF	8 d
XXI M K Å	M	5	1	M c	●	●		○	●					●	○L	IF	4 d
XXII J P A	M	8	10	M c		●		●	○				●	●		SDCT	3 d
XXIII C B	M	12	1	M c									●			SDCT	1 d

childhood Pitcock et al (12) compared exposures of less than 1800 r with more than 1800 r and found the former exposure often to result in a local recurrence whereas this was rarely seen with the latter exposure level Often, the treatments had been given over a 3-month period in their patients Bailey et al (1) on the other hand recommended a tumour dose of at least 3000 rad in involved areas in localized disease

In the present series local recurrence after radiation therapy was noted in 6 sites in 4 patients the recurrences being detected in 5 of the sites within 2 years and in one site 33 months after the initial treatment In 2 of the sites the recurrence occurred in connexion with generalization of the disease in 3 sites the recurrence was not the first new sign of disease after the initial treatment and in only one site (mediastinum patient XVII J B) was the recurrence the first new sign of disease after the first treatment

An attempt was done to evaluate the radiation response of Hodgkin s disease in children in the present series The radiation

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MALIGNANT LYMPHOMAS IN CHILDREN
A CLINICO-PATHOLOGIC RETROSPECTIVE STUDY

II *Non-Hodgkin's Lymphomas*

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ABSTRACT Garwicz, S., Landberg, T and Åkerman, M (Departments of Paediatrics, Radiotherapy Cytodiagnostics and Pathology University Hospital, Lund, Sweden). Malignant lymphomas in children—A clinico-pathologic retrospective study II. Non-Hodgkin's lymphomas. Acta Paediat Scand, 63:679 1974.—Data on 27 children with non-Hodgkin lymphomas are given. None had leukemia at the time of diagnosis. Twenty-one were boys. At microscopic review the lesions were classified according to Rappaport. All but one had diffuse lymphoma, which was lymphocytic in 13, histiocytic in 9 and of the Burkitt-type in 5. Pronounced differences thus exist between the morphology of non-Hodgkin's lymphomas in children and in adults. Extralymphatic manifestations were often seen, especially in lymphocytic lymphoma. This type also showed a marked tendency to leukemic transformation and accounted for 5 out of the 6 patients with leukemic transformation. Such transformation occurred within 6 months after the diagnosis. Treatment consisted of radiation therapy or chemotherapy or combination of the two. The results of radiation therapy indicate that the Burkitt-type tumours do not respond well to conventional fractionation. The survival rate in the present series was very poor Only 3 of the patients are still alive, whereas the remaining 24 have died mean 5 months after the beginning of treatment. Non-Hodgkin's lymphomas in children differ from non-Hodgkin's lymphomas in adults. Generalization is the rule and a more aggressive therapeutic approach is justified guided by the morphologic picture and utilizing radiation therapy and combination chemotherapy

KEY WORDS Non-Hodgkin's lymphomas

In a previous paper the authors (6) reported on 23 children with Hodgkin's disease They belonged to a group of 50 children with malignant lymphomas first seen at the Departments of Paediatrics and of Radiotherapy University Hospital Lund during the years 1944–72, the remaining 27 having non-Hodgkin's lymphomas The purpose of the present paper is to report on the 27 children with non Hodgkin's lymphomas Extrapolation of the figures given by the Swedish Cancer Registry (4) would indicate that during the period there may have been in the region served by the hospital about 53 new cases of non-Hodgkin's lymphomas in children Therefore the present series may not be representative of paediatric non Hodgkin's lymphomas in the region

Sex and Age

The sex and age for each patient is given in Table 1 There were 21 males and 6 females giving a ratio of 3.5 1 A male predominance has also been reported in other series varying from 1 9 1 (14) to 4.5 1 (13) averaging 2.6 1 in the compiled data (7) In the present

being cured whereas this may not be the case with 2 who have had extensions

Thirteen of the patients entered the study before 1968 and they have thus a possible follow-up of at least 5 years The 5 year survival rate for these is 6/13=46% Eleven of them had stage I or stage II disease at presentation and the 5 year survival rate for these 11 is 6/11=55% a figure of the same order as that found in a patient material of mainly adults from corresponding time interval (52% Landberg & Larsson 8) Of the 6 survivors 1 had Lymphocyte predominance 3 had Nodular sclerosing type and 2 had Mixed cellularity whereas of the 5 who had died 2 had Nodular sclerosing type and 3 had Mixed cellularity

In the series of Schnitzer et al (14) the Lymphocyte predominance type showed the best prognosis and in this respect the Nodular sclerosing type was not as favourable

Of 11 patients in the present series who had mediastinal presentation 7 are still alive 195–7 (mean 70) months after the beginning of treatment whereas 4 have died 51–4 (mean 30) months after the first treatment

Pitcock et al (12) stressed the good outlook in Hodgkin s disease in children Of their 44 patients about 30 per cent were alive after 5 years and of those with stage I disease 35% showed a 10 year survival Bailey et al (1) reported a 5 year survival-rate of 43% in 28 children with Hodgkin s disease and 4 stayed symptomfree for at least 10 years after the first treatment

Jenkin et al (7) found that stage by stage the mode of onset natural history or response to treatment did not differ in children from adults with the exception that in stage III the disease sometimes ran a more fulminating course in children

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eukemic transfor mation (mos. after agnosis)	Follow-up (mos.) after first treatment and status at end of follow-up	
No	231	a
No	24	a
No	4	a
No	31	d
1	13	d
1	8	d
No	7	d
No	6	d
3	6	d
No	6	d
No	5	d
No	4	d
No	4	d
No	4	d
No	3	d
No	3	d
No	3	d
2	3	d
No		d
No	2	d
No		d
No	2	d
No	1	d
No	1	d
1	1	d
6	1	d
No	0	d

phomas consisted of 30 children in whom evaluable microscopic material was available. At review 3 were excluded (2 having rhabdomyosarcoma and one having an undifferentiated sarcoma NUD) the remaining 27 patients form the present series. They were classified according to Rappaport (12) into lymphocytic well differentiated lymphocytic poorly differentiated lymphoma, histiocytic lymphoma mixed cell type lymphoma and stem-cell lymphoma including the Burkitt-type lymphoma. This classification may have therapeutic implications (5 10). Some of the non-Hodgkin s lymphoma cases especially those with a so-called starry-sky appearance were difficult to classify and above all it was difficult to distinguish between the stem-cell lymphomas and the lymphocytic poorly differentiated type. This was partly due to the fact that cytologic smears were not available in all these cases and in the patients with cytologic material the smears were only stained with hematoxylin and eosin. Analysing cytologic material (fine needle biopsies and touch preparations stained with both hematoxylin-eosin and Giemsa) may yield additional information to that obtained from ordinary histologic slides when classifying a non-Hodgkin s lymphoma.

Of the 27 patients (Table 1) 13 had lymphocytic lymphoma, 9 had histiocytic lymphoma, and 5 had lymphomas with a starry sky appearance. The cytologic material in these 5 cases showed besides large histiocytes with nuclear debris in the cytoplasm rather uniform tumour cells with a scanty cytoplasm and a round or oval nucleus with one or two fairly prominent nucleoli. On comparison with the first diagnosed case of Burkitt-lymphoma in Sweden (1) the cellular details in these 5 patients showed good resemblance. Six of the 13 patients with lymphocytic lymphoma were considered to have a well differentiated lymphoma whereas it was considered to be poorly differentiated in the remaining 7. No case of mixed cell type lymphoma was encountered in the present series.

The lymphoma was nodular in only one patient whereas it was diffuse in the remaining 26. In a series of 405 patients of all ages with non-Hodgkin s lymphomas Jones et al (10) found 44% to have nodular lymphoma, whereas a diffuse pattern was

Table 1 *Sex age clinical data microscopic type in initial biopsy and follow-up data for 27 children with non Hodgkin s lymphomas*

M=Male F=Female L=Lymphocytic lymphoma H=Histiocytic lymphoma B=Burkitt-type lymphoma, n=nodular d=diffuse w=well differentiated p=poorly differentiated ●=manifestations at first treatment O=later manifestations 1=chest wall 2=ileum and mesenteric lymph nodes 3=central nervous system 4=orbita, 5=spinal tumour 6=abdominal mass, a=alive d=dead

Patient	Sex	Age	Length of history (mos.)	Histol type in biopsy	Clinical localizations of disease: Neck right	Neck left	Axilla right	Axilla left	Mediastinum	Retroperitoneal	Groin right	Groin left	Spleen	Liver	Epipharynx-tonsil	Pleura	Skeleton	Soft tissue	Others
1 H A	M	8	1	H d			●												● 1
2 L. C G	M	11	1	H d															● 2
3 O K	M	3	1	H n							●								
4 A J	M	13		H d	●	●		●	●		O	O					O	O	O 1
5 W L.	F	1	1	L d w													●		
6 J B I	M	4	2	L d w	●	●	●		●			●		O		●			O 3
7 F M	M	6	3	B d	●	O	O	O											
8 L. A	M	10	1	H d	●	●									●			O	
9 N Å	M	13	2	L d p	●	●	●	●	●				O			●			
10 P F	M	5	2	H d		O			O	●	●	O		O					
11 P G	M	3	1	B d	O	O									●				
12 J M	F	4	1	H d	●	●									●		O		
13 C S Å	M	11	<1	L d w		O	O	O	●		O	O				●			
14 J I	M	12	<1	L d p		●													O 3
15 S B	F	14	2	L d p	●	●			●	O			O	O		O	O		
16 N J	M	8	0	L d w	●	●			●										● 5 O 3
17 S B	M	12	1	B d															
18 W J	M	5	4	L d p	●	●		●	●										
19 S M	F	3	2	L d w	●					O				O					O 4
20 J B	M	4	3	H d											●				● 5
21 G S	M	6	13	L d w															
22 S M	F	3		B d		●									O				
23 H K	F	5	3	B d		●				●					●	●			
24 K B	M	8	<1	L d p	●	●	●	●	●							●			● 1
25 F N	M	4	3	L d p										●				●	● 2
26 P K	M	3	6	H d														●	
27 S L.	M	7	6	L d p															● 6

series girls were usually younger than boys The age distribution indicates a peak incidence between 3 and 5 years and also suggests a bimodal incidence Thirteen children were less than 6 years of age the youngest being 16 months at the time of diagnosis The age distribution in the present series agrees with other reports which shows the same peak incidence and tendency to bimodal distribution (7 13)

Microscopy

Biopsy or post mortem tissue was obtained in all cases and from 1964 on also cytologic material (fine needle biopsies or touch preparations) in some cases All material was reviewed in detail by one of the authors (M Å) and categorized on the basis of recent concepts in histopathological classification

The initial material of non Hodgkin s lym-

the patients had at presentation also extra-lymphatic involvement in 10 of them the lymphoma was of the lymphocytic type in 3 of the histiocytic and in 2 of the Burkitt type

Seven patients with exclusively lymphatic involvement had at least 2 lymph node groups involved and in all 7 were the affected lymph node groups contiguous In 3 of these 7 the lymphoma was lymphocytic and in 4 it was histiocytic

Extensions were diagnosed in 14 patients In 5 the extensions were confined to the lymph node groups spleen or Waldeyer s ring, one of these 5 patients having lymphocytic lymphoma, 1 histiocytic lymphoma, and 3 Burkitt-type lymphoma. In 9 patients the extensions included extralymphatic sites 4 of these patients had lymphocytic lymphoma, 4 had histiocytic and one had Burkitt-type lymphoma

Thus it appears that at presentation lymphocytic lymphoma has a greater tendency to involve tissues outside the lymphatic system whereas the histiocytic lymphoma more often tends to be confined to the lymphatic system

Hematologic pre-treatment data were available in all patients Anemia was not common, only 3 patients had a hemoglobin value below 11 2 g/100 ml (=70% Sahli) 2 of them later developed leukemic transformation Leukopenia of less than 4 000/mm³ was noted in 5 patients two of them later developed leukemic transformation. The same 5 patients had granulocytes of less than 2 000/mm³ and 3 of them lymphocytes of less than 1 000/mm³ No other patient showed granulocytopenia or lymphopenia. Leukocytes between 4 000 and 10 000/mm³ were seen in 17 patients and values between 10 000 and 20 000/mm³ in 4 One of these 4 later developed leukemic transformation. One patient had a leukocytosis of 22 700/mm³ and subsequently showed bone marrow involvement Blood platelet counts were obtained in 11 patients all being normal Erythrocyte sedimentation rate in 23 patients was at most 10 mm/1 hr in 8 and at least 25 mm/1 hr in 10

Leukemic Transformation

Leukemic transformation as defined above was diagnosed in 6 patients occurring 1–6 months after diagnosis (Table 1) There were 5 boys and 1 girl (who was also the youngest patient) Five patients were at most 5 years of age and 1 patient was 13 years old All 6 had diffuse lymphoma which was histiocytic in one and lymphocytic in 5 in 3 of the 5 being poorly differentiated and in 2 being well differentiated In the whole series of 27 patients 9 had a follow-up of at least 6 months after the beginning of treatment and in all 3 of them with lymphocytic lymphoma leukemic transformation occurred whereas this was not seen in the 6 month survivors of other microscopic types

Leukemic transformation is a common event in the natural history of non-Hodgkin s lymphomas in children Its incidence varies largely in published series Bailey et al (3) reported leukemic transformation in 7 of 48 patients (15%) Sullivan (14) observed it in 41% of the patients and additional 24% of the children developed lymphomatous infiltration of the marrow demonstrated during life or at post mortem examination Jones & Klingberg (7) noted leukemic transformation in 9 out of 35 children (26%) Aur et al (2) in 9 out of 15 personal cases and in 33% of compiled data on 221 patients The highest incidence of leukemic transformation has been reported by Watanabe et al. (15) being 70% in 30 children with undifferentiated lymphoma, non-Burkitt-type It is difficult to compare all these figures because of lack of unifying histologic classification in the reported series The incidence of leukemic transformation is 22% in the present series but increases to 38% (5 out of 13) if only lymphocytic lymphoma is taken into account Sullivan (14) found that lymphoblastic

seen in the remaining 56% of their patients Their series included 19 children below the age of 15 and only one of these had nodular lymphoma whereas the remaining 18 had diffuse lymphoma

To the best of our knowledge there is only the report by Jones et al (10) dealing with the classification of Rappaport (12) in a series of non Hodgkin s lymphomas in children Bailey et al (3) in their report on 48 cases of non Hodgkin s lymphomas found 5 of the lymphocytic type 20 of the lymphoblastic type 7 of the reticulum cell type 14 of mixed cell type and 2 of the giant follicular type Jones & Klingberg (7) excluded cases of giant follicular lymphoma from their study and then classified 5 of the 45 cases as reticulum cell sarcoma and the remainder as lymphosarcoma Watanabe et al (15) mentioned that among 50 patients treated there were 30 undifferentiated lymphomas of non Burkitt-type 12 undifferentiated lymphomas of Burkitt-type and 8 histiocytic lymphomas No information was given regarding the nodular or diffuse pattern of the lymphomas Jones et al (10) found 7 of their patients below 15 years of age to have histiocytic lymphoma 4 to have mixed histiocytic lymphocytic 7 to have lymphocytic and 1 to have undifferentiated lymphoma

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Diagnostic Procedures

Staging procedures had been limited X-ray examination included chest X-ray in all patients and a skeletal survey in most. During the later period urography cavography and lymphography was performed in 5 4 and 2 patients respectively Scintigraphy of the liver was performed in 5 patients Three patients underwent laparotomy because of an abdominal mass

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Bone marrow aspiration was done in most patients at the time of diagnosis and also when peripheral blood or clinical findings suggested leukemic change. Multiple bone marrow aspirations or bone marrow biopsies were not performed The term leukemic transformation is used here denotes a total or almost total replacement of the bone marrow by abnormal lymphatic cells, being diagnosed during life It does not include patients in whom lymphoid cells suggestive of malignant lymphoma origin represented only a minor proportion of bone marrow cells or patients in whom a leukemic picture of the bone marrow was found only at post mortem.

Lumbar puncture was usually performed when clinical symptoms or signs suggested central nervous system involvement. CNS involvement was diagnosed when the cerebrospinal fluid contained abnormal lymphoid cells or there was evidence of invasive process, such as seizures cranial nerve involvement or involvement of the brain tissue

Clinical Data

The length of history (Table 1) was 1 month or less in 12 of the patients and was at least 4 months in only 4

As shown in the Table by far the most frequently involved site at presentation was the lymph nodes in the neck this being seen in 14 patients Axillary lymphadenopathy was seen initially in 6 patients and involvement of the lymph nodes in the groin in 3 Mediastinal lymphadenopathy was seen at presentation in 8 patients but retroperitoneal lymphadenopathy in only 2. No patient showed splenic involvement initially whereas one had involvement of the liver The epipharynx or the tonsillar region was involved initially in 5 patients the pleura in 5 the skeleton in one and soft tissues mainly in the form of subcutaneous tissue in 2 In 7 patients other tissues were involved at presentation namely the chest wall and the ileum with the mesenteric nodes and 2 patients had a spinal tumour and one had an abdominal mass

Twelve patients had at presentation only involvement of lymph nodes spleen or Waldeyer s ring In 3 of these 12 the lymphoma was lymphocytic in 6 it was histiocytic, and in 3 it was of the Burkitt-type Fifteen of

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Staging procedures had been limited X-ray examination included chest X-ray in all patients and a skeletal survey in most During the later period urography cavography and lymphography was performed in 5 4 and 2 patients respectively Scintigraphy of the liver was performed in 5 patients Three patients underwent laparotomy because of an abdominal mass

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Bone marrow aspiration was done in most patients a the time of diagnosis and also when peripheral blood c clinical findings suggested leukemic change Multipl bone marrow aspirations or bone marrow biopsies wer not performed The term leukemic transformation a used here denotes a total or almost total replacement o the bone marrow by abnormal lymphatic cells bein diagnosed *during life* It does not include patients i whom lymphoid cells suggestive of malignant lymphon origin represented only a minor proportion of bon marrow cells or patients in whom a leukemic pictu of the bone marrow was found only at post mortem

Lumbar puncture was usually performed when clin cal symptoms or signs suggested central nervous sy tem involvement CNS involvement was diagnose when the cerebrospinal fluid contained abnormal lym phoid cells or there was evidence of invasive proces such as seizures cranial nerve involvement or involv ment of the brain tissue

Clinical Data

The length of history (Table 1) was 1 mont or less in 12 of the patients and was a least 4 months in only 4

As shown in the Table by far the mos frequently involved site at presentation wa the lymph nodes in the neck this being see in 14 patients Axillary lymphadenopath was seen initially in 6 patients and involve ment of the lymph nodes in the groin in 3 Mediastinal lymphadenopathy was seen a presentation in 8 patients but retroperi toneal lymphadenopathy in only 2 No pa tient showed splenic involvement initially whereas one had involvement of the liver The epipharynx or the tonsillar region wa involved initially in 5 patients the pleura in 5 the skeleton in one and soft tissues mainly in the form of subcutaneous tissue in 2 In 7 patients other tissues were involved at presentation namely the chest wall and the ileum with the mesenteric nodes and 2 patients had a spinal tumour and one had an abdominal mass

Twelve patients had at presentation only involvement of lymph nodes spleen or Waldeyer s ring In 3 of these 12 the lymphoma was lymphocytic in 6 it was histiocytic and in 3 it was of the Burkitt type Fifteen of

10 fractions over 2 to 4 weeks had been given and only few patients received larger absorbed doses The short median survival does not warrant an investigation of the radiation response of the tumours and only a remark on the results of radiation therapy of the Burkitt-type lymphomas will be given. The Burkitt-type tumours did not respond well to conventional fractionation with one fraction per day Two of the patients with Burkitt-type tumours have a very short follow-up whereas 2 (7 F M and 11 P G) had a follow-up of 7 and 5 months respectively In both these patients the tumours grew during radiation therapy which did not seem to influence the tumours at all The absorbed radiation dose in the target was calculated for these 2 patients and expressed for cobalt-60 radiation quality (RBE=0.85 of the unit value) and was for patient 7 F M 4800–5600 rad given in 34 fractions over 119 days in split-course in three series and for patient 11 P G 4900 rad given in 34 fractions over 70 days in 2 series The calculated NSD- and TSD-values were for patient 7 F M 1200–1400 ret and 2100–2300 ret, respectively and for patient 11 P G 1300 ret and 2100 ret respectively In no other patient in the present series was such an apparent resistance to conventionally fractionated radiation therapy seen Norin et al. (11) found in children from tropical Africa with Burkitt's lymphoma that radiation therapy given with absorbed doses of up to 5100 rad in 25 fractions over 30 days (NSD=1600 ret and TSD=2350 ret) with one fraction a day yielded most disappointing results When a superfractionated treatment with 3 fractions a day was used consistently better results were obtained for the local effect on the tumour The authors consider the unusual short generation time in Burkitt's lymphoma to be a possible explanation.

In a further 2 patients in the present series a local recurrence was seen after radiation therapy occurring after 2–3 months. The absorbed radiation dose in these patients (16 N J and 19 S M) may be calculated to have been of the order 900 rad in 11 fractions over 13 days (NSD=400 ret and TSD=550 ret) and 2500 rad in 14 fractions over 15 days (NSD=1000 ret and TSD=1350 ret) respectively

Survival

Three patients are still alive (Table 1) 231 24 and 4 months after the inception of treatment, whereas the remaining 24 have died after 0–31 (median 3 mean 5) months. All 3 survivors are boys

All 3 survivors had histiocytic lymphoma one of them of the nodular pattern Of patients who have died, those having lymphocytic lymphoma had a survival after the first treatment of 0–13 (median 3) months whereas those with histiocytic lymphoma had a survival of 1–31 (median 5) and those with Burkitt-type tumours 1–7 months

In the present series histiocytic lymphoma thus carried the best prognosis

One patient is a long-term survivor and can reasonably be regarded as being cured. patient 1 H A. (Table 1) male aged 8 who presented with a one-month history of an ulceration in the skin ventrally in the right axilla and enlarged lymph nodes in the right axilla. Biopsy proved a diffuse histiocytic lymphoma, and radiation therapy was given with one ventral field (170 kV HVL 1 0 mm Cu FSD 60 cm) 3000 R were delivered in 10 fractions over 48 days in split-course with an interval of 37 days between the two series The absorbed radiation dose in the target, expressed for cobalt 60 radiation quality may be calculated to have been of the order 3300–2200 rad (NSD=1250–850 ret and TSD=1900–1250 ret) The ulceration healed and the enlarged lymph nodes regressed Eight years later an aseptic necrosis of the 6th rib developed The rib was excised no tumour could be found histologically The patient is well more than 19 years after the treatment

and lymphocytic lymphosarcoma were the original diagnoses in equal numbers of children in whom leukemia developed none of the 2 children with reticulum cell sarcoma in their series developing leukemic transformation The influence of the histologic type of lymphoma on the rate of marrow infiltration has been demonstrated by Jones et al (8) Patients with histiocytic lymphoma had initially bone marrow involvement in only 5% as contrasted with 30% in the lymphocytic type Marrow involvement was not influenced by the nodular or diffuse patterns of the lymphoma In the same study it was demonstrated that marrow biopsy was superior to aspiration in detecting bone marrow involvement

In our series leukemic transformation occurred 1 to 6 months after diagnosis In the material of Jones & Klingberg (7) the time from diagnosis to leukemia was 2 4 months Sullivan (14) reported that the duration of disease at the time of conversion to leukemia was 2 5 to 15 months with an average of 7 Taking into account the length of history in the present material the leukemic transformation occurred 3 to 12 months from the onset of symptoms Of the 6 patients who had shown leukemic transformation 5 had at the time of presentation involvement of extralymphatic tissue (Table 1) Sullivan (14) could not find any relationship between primary site of involvement and extent of disease on the one hand and the subsequent leukemic transformation on the other and Jones et al (8) stated that no characteristic pattern of laboratory or clinical findings indicative of marrow involvement had emerged from their study but there was a correlation with advanced stage of the disease On the other hand Watanabe et al (15) observed leukemic transformation in 10 out of 11 patients with mediastinal involvement and in only 2 out of 10 children with an abdominal mass Of the 6 leukemic transformers in the present series 3 had mediastinal involvement whereas the remaining 6 with mediastinal lymphadenopathy did not show leukemic transformation

Central nervous system involvement was seen in 3 patients one of them also having leukemic transformation In 2 the lymphoma was of the lymphocytic type and in one of the Burkitt-type Watanabe et al (15) reported the frequency of meningeal involvement to be as high as 48% in patients with bone marrow involvement This figure is of the same order as the frequency of central nervous system involvement in acute lymphoblastic leukemia in childhood

Treatment

Twenty-two patients received radiation therapy as the initial treatment in 6 of them was also chemotherapy given In 3 patients with extralymphatic spread was chemotherapy alone the initial treatment and in one further patient the initial treatment consisted of resection of an intestinal tumour followed by chemotherapy One patient died before treatment was started Eight patients received no further radiation therapy or chemotherapy whereas 18 did

No special policy existed regarding type of chemotherapy used one reason being that the patient material spans over a 29 year period Ten patients received chemotherapy as part of the initial treatment or as sole therapy Single drug was used in 4 patients 3 children received sequential chemotherapy and 3 others were treated with a combination of drugs Usually Cyclophosphamide was used in 3 patients as the only agent in an other 3 as the first drug in sequential chemotherapy and in one child in combination with other drugs Vincristine or Vinblastine were the next most often used drugs In leukemic transformation combination therapy including Prednisolone and antimetabolites was used in some patients

Radiation therapy was usually given with 170 kV roentgen rays or with cobalt-60 Usually relatively small absorbed radiation doses of the order 1 800–2 500 rad in about

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BRAIN GROWTH IN CHILDREN WITH KWASHIORKOR

A Study using Head Circumference Measurement Transillumination and Ultrasonic Echo Ventriculography

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ABSTRACT Engsner G., Habte D., Sjögren, I. and Vahlquist, B. (Ethiopian Nutrition Institute and the Ethio-Swedish Pediatric Clinic, Addis Ababa Ethiopia and the Department of Paediatrics, University Hospital Uppsala, Sweden). Brain growth in children with kwashiorkor A study using head circumference measurement, transillumination and ultrasonic echo ventriculography Acta Paediatr Scand 63, 1974.—Brain growth was studied in 53 children with kwashiorkor and marasmic kwashiorkor by the simultaneous use of head circumference measurement, transillumination and ultrasonic echo ventriculography The results of examinations on admission showed that the head circumference was reduced but probably slightly less so than in marasmic children. The transillumination findings were distinctly abnormal; bearing the age difference in mind, the abnormality was more pronounced than in the cases of marasmus. The lateral ventricle index, calculated from echo ventriculograms, was increased, which was not the case in marasmus. A systematic follow-up study for 6 months of 10 patients showed a gradual and complete normalization of the transillumination and echo ventriculographic findings. The interpretation of the results, taking into consideration also the possible sources of error is discussed.

KEY WORDS: Brain, kwashiorkor transillumination, echo encephalography head circumference

Brain growth in children with kwashiorkor has been evaluated mainly by measuring head circumference This is not however a very satisfactory method in this kind of clientele (21 30) Information on brain weight accurately determined in children with well-defined kwashiorkor is still sorely lacking.

Only two reports provide data of some interest in this respect (1 9)

As part of the work done at the Ethiopian Nutrition Institute (ENI) Addis Ababa, studies of brain growth in young children were carried out between 1969 and 1972 (7 8) The aim of the study to be presented in this article was two-fold

(1) To measure the brain size in children with kwashiorkor by simultaneous recording of head circumference and transillumination and echo ventriculographic findings

(2) To demonstrate whether or not in children with kwashiorkor appreciable improvement in brain size takes place during nutrition rehabilitation.

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Table 1 *Anthropometric data in children with kwashiorkor and marasmic kwashiorkor*

Age group (months)	n	Weight (kg)	Weight/age % standard	Length (cm)	Length/age % standard	Weight/length % standard	Arm circumference (cm)	Triceps skinfold (mm)	Head circumference (cm)
Kwashiorkor									
12–17	4	7.40 [1.04]	71 [5]	70.9 [7.6]	91 [4]	83 [6]	11.4 [1.3]	6.6 [0.9]	45.4 [1.7]
18–23	9	7.82 [0.64]	75 [5]	74.7 [4.1]	89 [10]	81 [11]	11.6 [1.4]	6.6 [illegible]	46.2 [2.9]
24–29	7	9.13 [1.68]	77 [11]	78.1 [5.6]	88 [7]	86 [15]	[illegible] [1.5]	8.4 [illegible]	47.3 [illegible]
30–36	5	10.51 [1.06]	75 [4]	83.5 [10.8]	89 [9]	90 [9]	[illegible] [1.6]	7.5 [2.7]	47.4 [illegible]
Marasmic kwashiorkor									
12–17	7	5.97 [0.64]	55 [5]	68.9 [4.8]	88 [8]	71 [16]	9.6 [1.1]	4.9 [2.1]	44.5 [3.8]
18–23	10	6.74 [0.34]	56 [6]	[illegible] [4.8]	87 [6]	69 [8]	9.9 [1.1]	[illegible] [1.6]	45.8 [2.1]
24–29	6	6.97 [0.94]	55 [7]	74.5 [7.8]	84 [9]	77 [5]	10.0 [1.0]	5.8 [1.6]	45.6 [illegible]
30–36	5	8.02 [0.98]	57 [4]	76.1 [4.4]	81 [7]	78 [8]	9.9 [0.8]	5.1 [1.6]	45.9 [0.9]

Figures in brackets are two standard deviations.

RESULTS

Cross sectional study

Anthropometry The anthropometric data for the children with kwashiorkor and marasmic kwashiorkor are presented in Table 1

Head circumference The data presented in Fig. 1 *a* and 1 *b* show individual values for the children with kwashiorkor which are throughout at or below the 50% percentile of the Swedish norms (13) There is a tendency for children with marasmic kwashiorkor to manifest lower values than the children with kwashiorkor but the difference does not reach statistical significance in this material ($p>0.05$)

Transillumination The transillumination findings presented in Fig. 2 show an increased translucency with no difference between the two types of kwashiorkor (marasmic or not) The difference from the controls is highly significant ($p<0.001$)

Ultrasonic echo ventriculography The lateral ventricle indices presented in Fig. 3 are significantly ($p<0.01$) above normal for the children with kwashiorkor as well as for

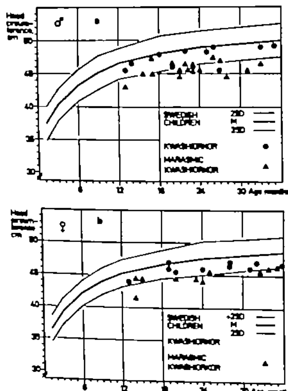


Fig. 1 (*a*) Head circumference related to age. Children with kwashiorkor and marasmic kwashiorkor compared with normal range (13). Cross-sectional study. Boys only (*b*) Head circumference related to age. Children with kwashiorkor and marasmic kwashiorkor compared with normal range (13). Cross-sectional study. Girls only

MATERIAL

The definitions used were those given in the Jamaica Declaration (6) i e oedematous children with a weight for age between 60 and 80% of the standard (1?) were classified as suffering from kwashiorkor and those with a weight for age below 60% as suffering from marasmic kwashiorkor

Only patients with the classical clinical picture and laboratory findings and with no other demonstrable causes of oedema (kidney disease etc.) were included in the material

The total number of children suffering from kwashiorkor and marasmic kwashiorkor examined was 53 All the children were hospitalized at the Ethio-Swedish Pediatric Clinic (ESPC) Addis Ababa.

The *examinati n plan* was as follows

Baseline examinations were performed within ?4 hours of admission (cross-sectional study)

Follow up examinations were performed every fortnight during the stay at the hospital and monthly after discharge (longitudinal study ")

For various reasons (death of children breaking off schedule of treatment and/or follow-up) only 10 children could be examined according to plan during a period of 6 months

Nutrition rehabilitation program

Nutrition rehabilitation consisted of the provision of a high protein (4 g/kg body weight) high-calorie (150 cal/kg body weight) liquid diet made up of a mixture of a cow s milk protein concentrate (Casilan Glaxo) non-fat milk vegetable oil sugar potassium chloride and water This was usually given via a nasogastric tube during the first few days because of anorexia. Semi-solid food was introduced usually during the second week and whole milk was substituted for the high-protein mixture during the third week. Appropriate vitamins and antibiotics were administered on liberal indications. Progress assessment included daily weight weekly total serum protein and fortnightly serum albumin determinations.

After discharge the children were examined on a monthly basis and each time the mothers were provided with 3 kg of Faffa (a protein-rich low-cost weaning food produced at the ENI) If correctly used the daily supplementary intake should have been 100 g, containing ca. 20 g of protein 340 calories and in addition minerals and vitamins

METHODS

All the children were examined by one and the same pediatrician (G E.) Interviews with the children s guardians mostly their mothers were carried out with an Ethiopian nurse or health officer acting as interpreter The nurse or health officer also acted as an assistant at the examinations.

Anthropometry Standard anthropometric data, including body weight length arm circumference head circumference and skinfold thickness (triceps) were recorded For details, see WHO Monograph Series No. 53 (1?) Scales and tapes were regularly checked A Harpenden caliper was used.

Head circumference was of particular importance in these studies. Great care was taken to obtain accurate and reproducible results The measurement was made with a steel tape to the nearest 0 1 cm The tape was placed so as to measure the greatest occipito-frontal circumference

Transillumination The transillumination examinations were performed by using a transillumination lamp of the Oculus type with a small 25 Watt (7.5 V) lamp and a point scale fixed to the rim by a black rubber adapter The results of the examinations were expressed in scale points, according to the method described by Sjogren & Engsner (?6).

Normally newborn infants should transilluminate fronto-temporally up to scale point ? or less and parieto-occipitally up to scale point 1 or less. Children aged more than 1 months should not illuminate the scale plate at all

Ultrasonic echo ventriculography The size of the lateral ventricles was measured by ultrasonic echo (22, 23). The echo encephalograph used was a Siemens apparatus (Kraut Kramer system) with a Polaroid Land camera for instant recording of the oscillographic tracings The probe used had a frequency of ? megacycles per second and a diameter of 4 mm. Liquid paraffin was used as a contact medium between the head and the probe The head was not shaved prior to the examination

The summarized width of the right and left lateral ventricles (T) was expressed in relation to the diameter of the child s head (D) as a lateral ventricle index I) i e $T/D=I$

Normally this index should not exceed 0.33 or 33 per cent (M+1 SD) of the diameter of the head in newborn babies and 0 29 in children aged 12 months (25)

Discussion of the methods

Some difficulties in the interpretation of the recorded data are discussed in another paper of this series dealing with the situation in cases of marasmus (8) They refer to such factors as *thinning of scalp and bone* as well as *demineralization of bone*

The presence of *oedema* in the scalp may exaggerate the head circumference and the transillumination area, whereas the lateral ventricle index should hardly be affected According to Robinow (21) oedema may increase the head circumference by as much as 1.5–. cm. However this statement does not agree with the observations in our material. In 32 of the 53 children with kwashiorkor or marasmic kwashiorkor subjected to repeated examinations, the reduction of the head circumference 2 weeks after admission when visible oedema had regularly disappeared amounted to only 0.0–0.8 cm with a mean of 0.5 cm.

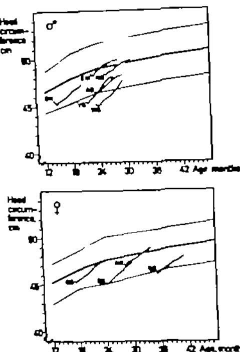


Fig 5 Head circumference related to age Children with kwashiorkor and marasmic kwashiorkor Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation. Individual values compared with normal range (13)

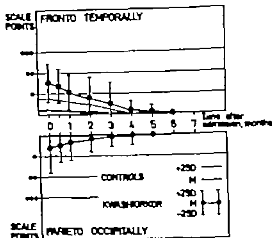


Fig 6 Head transillumination related to age Children with kwashiorkor and marasmic kwashiorkor compared with non-privileged Ethiopian children Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation. Boys and girls combined

brain weight for oedema Now the question of brain oedema in kwashiorkor is surprisingly enough far from clear Reference is often made to the monograph by Trowell Davies & Dean (29) However this contains only a brief note in passing (As previously mentioned there is often oedema and congestion of the brain at autopsy) and does not give any factual information on the matter

Garrow et al. (9) found in 2 cases of kwashiorkor (infants 6 and 16 months old) an increased water content (87% as compared with 83.5% as the mean of 4 children who were malnourished but had no oedema) But this difference does not seem to be corroborated in a more recent study (31) Maybe we are faced with a situation similar to the one observed in protein-calorie-deficient animals From studies on pigs, Platt et al concluded (20) that, "although the consistency of the unfixed brains gave the impression of being wetter and less firm than normal the water content was similar to that found in the brain of normal age controls

With respect to *head circumference* the results of our studies indicate a significant reduction on admission more pronounced in marasmic kwashiorkor than in kwashiorkor

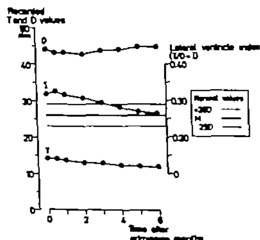


Fig 7 Ultrasonic echo encephalography Indices *T* (transverse width of the two lateral ventricles), *D* (external diameter of head) and *I* (lateral ventricle index *T/D*) related to age. Children with kwashiorkor and marasmic kwashiorkor Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation. Boys and girls combined.

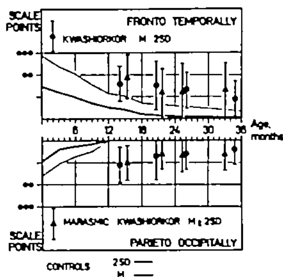


Fig 2 Head transillumination related to age Children with kwashiorkor and marasmic kwashiorkor compared with a control group of Ethiopian children (7) Cross-sectional study Boys and girls combined

those with marasmic kwashiorkor Thus there is a moderate increase of the size of the lateral ventricles

Longitudinal study

The results of the *anthropometric measurements* from the follow-up examinations for 6 months or more in the 10 cases (7 of kwashiorkor and 3 of marasmic kwashiorkor) are given in Fig 4

A clear-cut tendency to catch up in *head*

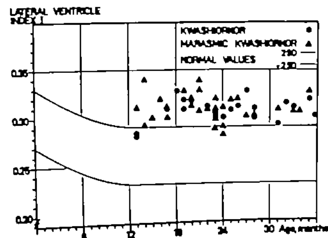


Fig 3 Ultrasonic echo encephalography Lateral ventricle index (*I*) related to age Children with kwashiorkor and marasmic kwashiorkor compared with normal range (25) Cross-sectional study Boys and girls combined

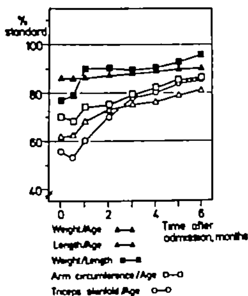


Fig 4 Anthropometric data expressed as means of percentage standard (17) Children with kwashiorkor and marasmic kwashiorkor Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation Boys and girls combined.

circumference in relation to age was noted (Fig 5)

A gradual normalization of the *transillumination* findings was noted (Fig. 6) and after 4 months treatment the values had come close to zero i e quite normal values for the age groups

The *echo ventriculographic findings* also indicated gradual normalization with a decrease in the lateral ventricle indices (Fig. 7). At 6 months after admission to hospital the lateral ventricle indices had completely returned to normal

DISCUSSION

With respect to *brain weight* at autopsy in children with kwashiorkor no well-defined material has been presented so far Brown published from Uganda the brain weights of a large material of children diagnosed at autopsy as suffering from malnutrition (1) In the age groups 1–4 years which represented some degree of kwashiorkor with isolated exceptions the mean weights were 15–20% below the reference group The difference might have been even greater if it had been necessary or possible to correct the

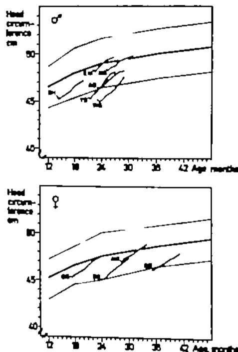


Fig 5 Head circumference related to age. Children with kwashiorkor and marasmic kwashiorkor. Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation. Individual values compared with normal range (13).

brain weight for oedema" Now the question of brain oedema in kwashiorkor is surprisingly enough far from clear Reference is often made to the monograph by Trowell, Davies & Dean (29) However this contains only a brief note in passing (As previously mentioned there is often oedema and congestion of the brain at autopsy ') and does not give any factual information on the matter

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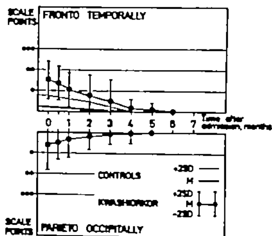


Fig 6 Head transillumination related to age. Children with kwashiorkor and marasmic kwashiorkor compared with non-privileged Ethiopian children. Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation Boys and girls combined.

With respect to *head circumference* the results of our studies indicate a significant reduction on admission more pronounced in marasmic kwashiorkor than in kwashiorkor

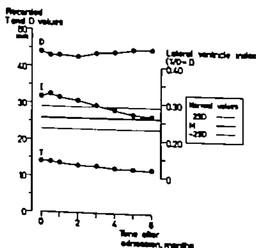


Fig 7 Ultrasonic echo encephalography Indices *T* (transverse width of the two lateral ventricles) *D* (external diameter of head) and *I* (lateral ventricle index, *T/D*) related to age. Children with kwashiorkor and marasmic kwashiorkor Longitudinal study of 10 cases examined at monthly intervals during nutrition rehabilitation. Boys and girls combined.

The mean size of reduction corresponds well to the figure of 4% given by Dean (4)

There is otherwise a scarcity of data most of the head circumference measurements published refer to PCM of the marasmus type Several longitudinal studies of children with kwashiorkor lack head circumference measurements from the initial hospitalization period At follow-up after a number of years the head circumference showed no (10 14) or only modest (2) deviation from normal

During nutrition rehabilitation a catch-up growth was observed Because of possible simultaneous changes in thickness of scalp and bone (8) it is difficult to decide to what extent a faster-than normal growth of skull cavity circumference has taken place

With respect to *transillumination* we have not been able to find any earlier report dealing with children with kwashiorkor Our studies yielded clearly abnormal results In healthy children it is generally accepted that transillumination ceases to occur at the age of 12 months In the group of non privileged Ethiopian children (with no or only mild PCM) introduced for comparison weak trans illumination could be observed also after 18 months of age However in our group of children with kwashiorkor very marked trans illumination could be observed all the way up to 36 months Clearly there were children with kwashiorkor who manifested only slight translucency after the age of 18 months but the mean values on admission were through out distinctly abnormal

What is the explanation of the abnormal transillumination in the acute phase of kwashiorkor? In neuropediatric practice abnormal transillumination is synonymous if pronounced hydrocephalus can be excluded with subdural or subarachnoidal accumulation of fluid mostly subdural effusion However in cases of kwashiorkor special caution is warranted in the interpretation of the findings

Thinning and/or demineralization of the bone thinning and/or oedema of the scalp tissues and crew-cut hair may all contribute to a non-specific increase of transillumination viz to the persistence of translucency at an age when it should normally have vanished

For various reasons (persistence of abnormal transillumination long after oedema of the scalp had disappeared much more marked transillumination in kwashiorkor than in marasmus) it seems reasonable to assume that in the kwashiorkor children there was an abnormal accumulation of fluid in the subdural/subarachnoidal space For ethical reasons however no attempt was made to analyse this further using needle aspiration

The findings at *ultrasonic echo ventriculography* verified on a larger material the observations made earlier by the authors (30) namely that there is an increase of the lateral ventricle index in the acute stage of the disease The longitudinal study now carried out made it clear that this abnormality is transitory in character during nutrition rehabilitation over a period of 6 months a gradual decline of the index took place ending in normal values

The volume for all four brain ventricles in healthy adults has been given as ca 16-20 ml (3) A close correlation has been noted between the lateral ventricle width as measured from echo ventriculograms and the size of the ventricles as measured from pneumograms (15 24)

A possible exception may be the situation in infants below 6 months in whom the higher index normally observed has been postulated to have its origin in a thick septum pellucidum (16) The midline echo in our children who were all above 12 months of age did not show any recurring changes (e g abnormally broadened multispiked midline echoes)

A note made in passing by Platt et al (20) is of some interest in this context In their studies on protein-calorie-deficient animals (pigs) they noted that in some animals there appeared to be a very slight enlarge-

ment of the lateral ventricles the cerebrospinal fluid pressure was not measured and no papilloedema was found in any animal in these experiments

After the completion of our field work we were made aware of the existence of a study in Brazil (17 in Portuguese) involving also the taking of pneumograms in children with kwashiorkor "Cortical atrophy in variable degrees was an almost regular finding in the material of 15 cases X-ray diagnosis of cortical atrophy in so far as it is not very pronounced is often liable to errors However the fact that repeated examinations with the same technique after 12 months in 12 out of 15 children showed a complete or almost complete return to normal argues that it was a true transitory abnormality similar to the one disclosed in our study by transillumination Likewise the transitory changes of lateral ventricle width in our studies are matched by observations in the Brazilian study of slight-to-moderate enlargement of the lateral ventricles in 11 of the 15 children examined Again with two exceptions normalization had taken place at the follow-up study 12 months later

What can be the cause of the transitory increase in subdural/subarachnoidal space and in lateral ventricle size in cases of kwashiorkor? In malnourished children with oedema, Smith (27) found a mean value for the total body water (TBW) of 82 18% Within 35 days of the loss of oedema, this value had declined to 69 84% A further slow decline continued over several months Could the slow eventually complete regularization of the transillumination and echo ventriculographic findings in our series be explained as a parallel phenomenon to the TBW situation? We do not believe that this is the case After all the disappearance of visible oedema and the major decline of TBW proceeds much faster than the decline of transillumination and lateral ventricle width

There seems little reason for thinking that gross abnormalities such as sinus thrombosis and/or subdural effusion can be regularly involved and reversible in cases of kwashiorkor But what about metabolic changes due to hypovitaminosis particularly of vitamin A? Severe signs of vitamin A deficiency often with frank xerophthalmia are of common occurrence in cases of kwashiorkor The explanation may be a true nutritional deficiency of the vitamin (which is of common occurrence in Ethiopia) and/or an impaired hepatic release of vitamin A due to defective production of retinol-binding protein (28) Experiments on animals have clearly shown that not only *hyper* but also *hypo*vitaminosis A of some duration and intensity will block the free circulation of the cerebrospinal fluid and thereby cause increased intracranial pressure (5 11 18 19) We did not measure the liquor pressure in our children with kwashiorkor but there were no obvious clinical signs of increased intracranial pressure

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THE EFFECT OF DISACCHARIDES ON THE HYPERLACTACIDAEMIA OF GLUCOSE-6-PHOSPHATASE DEFICIENT CHILDREN

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ABSTRACT Fernandes, J (Department of Paediatrics, Sophia Children's Hospital and Neonatal Unit, Erasmus University Rotterdam, The Netherlands). The effect of disaccharides on the hyperlactacidaemia of glucose-6-phosphatase-deficient children Acta Paediat Scand, 63:695 1974.—Two unrelated children with hepatic glycogenosis due to glucose-6-phosphatase deficiency were investigated as regards the influence of dietary disaccharides on their hyperlactacidaemia. The disaccharides were administered orally as 10% solutions hourly during 12 hours in an A–B–A sequence, i.e. maltose-lactose-maltose or maltose-sucrose-maltose. Each disaccharide was given in repeated doses. Blood lactate levels increased during the lactose or sucrose feedings and decreased during the maltose feedings, while normoglycaemia was maintained throughout the experiments. It is concluded that lactose and sucrose should be restricted in the diet of children with a glucose-6-phosphatase deficiency

KEY WORDS: Glycogen storage disease glucose-6-phosphatase deficiency and hyperlactacidaemia

The hyperlactacidaemia of patients deficient in glucose-6-phosphatase is known to be caused by an overproduction of lactate in the liver (9 8) Lactate production decreases after ingestion of glucose (8) and increases after ingestion of galactose or fructose (1) Administration of the latter two hexoses also causes hypoglycaemia in children with a glucose-6-phosphatase deficiency because glucose formation from galactose or fructose is blocked We therefore recommended a restriction of the galactose and fructose in the diets of these patients (1) It does not necessarily follow that the disaccharides lactose and sucrose should be restricted too. Lactose or sucrose ingestion does not lead to hypoglycaemia since this is prevented by the glucose component of these disaccharides Thus a hypoglycaemia-induced hyperlactacidaemia is not to be expected. An increased lactate production from the galactose or fructose component, however could still occur We therefore studied the blood lactate levels during oral administration of lactose or sucrose as compared with maltose in two patients with a glucose-6-phosphatase deficiency

METHODS AND RESULTS

The two patients were unrelated one-year-old girls. They showed similar abnormalities, i.e. hepatomegaly tendency to hypoglycaemia, lacticacidosis and hyperlipidaemia. Glucose-6-phosphatase activities (2) measured in the liver biopsies of the patients, were 4.3 and 0.9 nmoles P_i/min/mg protein respectively (normal range 24 0–93 0 nmoles P_i/min/mg protein, $n=6$). Blood glucose was determined with glucose oxidase (6) blood lactate with lactate dehydrogenase (4)

Maltose, lactose and sucrose were administered orally as 10% solutions hourly during 12 hours. Blood glucose and lactate were determined prior to each sugar feeding. Every experiment was started 3 hours after the last

Dr J F Koster Department of Biochemistry performed the enzyme assays

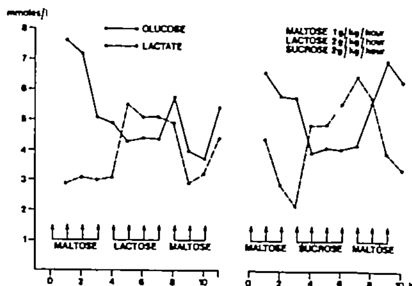


Fig 1 Glucose and lactate in the blood after repeated oral administration of different disaccharides to patient no 1 with a glucose-6-phosphatase deficiency

normal feeding. The disaccharides were administered in an A–B–A sequence: maltose–lactose–maltose or maltose–sucrose–maltose

In one experiment the doses of sucrose were equal to the doses of maltose (Fig. 3 right) In the other experiments the doses of lactose and sucrose were twice the doses of maltose (Fig. 1 2 and 3 left) in order to administer equal amounts of glucose and thus maintain constant blood glucose levels. Major alterations of the glucose levels did occur nevertheless (Fig. 1) though a normoglycaemic state was maintained throughout all experiments In all experiments blood lactate concentrations increased after the replacement of maltose by lactose or sucrose the reverse phenomenon occurring after the reintroduction of maltose The largest increments of the blood lactate concentrations were observed during the sucrose feedings

DISCUSSION

Some factors are known to influence the hyperlactacidaemia of patients with a glycogenosis caused by a deficiency of glucose-6-phosphatase These factors are the frequency of the meals the carbohydrate content of the diet and the nature of the carbohydrates Kelsch & Olivier (7) changed the frequency of the meals and the nature of the carbohydrates simultaneously in one patient After the replacement of a normal home diet with a high content of sucrose by an isocaloric high-glucose diet in which the meals were given every three hours the hyperlactacidaemia and hyperlactaciduria decreased considerably The simultaneous alteration of two factors however makes the evaluation of the effect of each separate factor upon the lactate production impossible

The carbohydrate content of the diet cannot be varied much because a low-carbo-

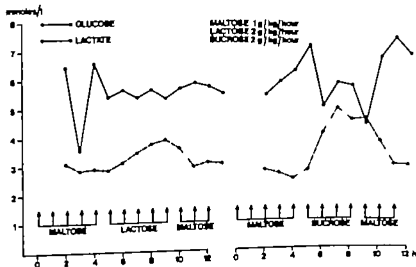


Fig 2 Glucose and lactate in the blood after repeated oral administration of different disaccharides to patient no 2 with a glucose-6-phosphatase deficiency

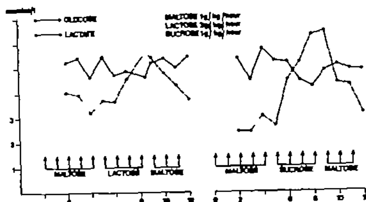


Fig 3 Glucose and lactate in the blood after repeated oral administration of different disaccharides to patient no with a glucose-6-phosphatase deficiency

hydrate diet entails an increased risk of hypoglycaemia. Minor differences in the carbohydrate content of the diet did not greatly influence the hyperlactacidaemia and hyperlactaciduria of the two patients (unpublished results)

As regards the nature of the carbohydrates we found that lactose and sucrose had the same untoward effect on blood lactate levels as galactose and fructose

The blood lactate increase during lactose or sucrose administration to our patients might be explained as follows The galactose and the fructose components of lactose and sucrose respectively are almost exclusively and quite rapidly metabolized in the liver

Galactose can either be converted into glycogen or into lactate The formation of glycogen may well be hampered by a defective activity of glycogen synthetase due to hypoinsulinaemia (3 5) and a high glycogen content of the liver (11) The conversion into lactate on the other hand could be enhanced by the initial phosphorylation of galactose with a concomitant increase of the AMP level which in its turn stimulates phosphofructokinase activity (10) and thus glycolysis

Fructose is preferentially metabolized into lactate by a pathway which bypasses the phosphofructokinase reaction, the rate-limiting step of glycolysis

As for maltose its glucose components can be metabolized to a greater extent extrahepatically Glucose is more slowly phosphorylated in the liver and lactate production is less stimulated than in the case of the other two hexoses

On the basis of our findings we recommend a restricted lactose and (especially) sucrose intake in the diets of children with a glucose-6-phosphatase deficiency

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SERUM LEVELS AND RENAL EXCRETION OF DIGOXIN DURING MAINTENANCE THERAPY IN CHILDREN

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ABSTRACT Iisalo, E. and Dahl, M. (Departments of Pharmacology and Paediatrics, University of Turku, Turku, Finland). Serum Levels and Renal Excretion of Digoxin during Maintenance Therapy in Children. Acta Paediatr Scand, 63:699 1974.—To exclude an increased excretion of digoxin as a possible reason for higher dose requirement in children a radioimmunoassay of digoxin in the serum and the 24 hr urine was carried out in 26 children, mainly infants, with congenital heart disease. This procedure was repeated on two consecutive days, in total 64 times during digoxin maintenance therapy

The daily digoxin dose per kilogram of body weight in these small children was twice as high as that used in adults. The steady state level of serum digoxin corresponded approximately to that of adults. A few fairly high digoxin levels were, however measured in infants without any signs of digitalis toxicity

The daily excretion of digoxin in the urine during maintenance therapy was approximately the same in all age groups and on an average 47 per cent of the daily dose. This percentage is somewhat lower than that found in adults. The low renal excretion of digoxin in one newborn caused high serum digoxin levels.

The higher dosage requirement of digoxin per kilogram of body weight in children as compared to adults cannot be explained with differences in the renal excretion of digoxin.

KEY WORDS: Neonates, small children, serum digoxin, renal excretion of digoxin

It is well-known that in the treatment of cardiac insufficiency in children a higher dose of digitalis glycosides is needed per kilogram of body weight than in adults In a recent study (8) we showed that to achieve the same serum digoxin level as in adults a dose twice or three times as high per kilogram of body weight is needed for children. The reason for this is not known. In 1972 Krasula et al (12) suggested a possibility that infants have a more rapid renal excretion of digoxin than older children and adults We have therefore studied the excretion of digoxin in children with congenital heart disease in the steady state of the treatment and compared it with the serum digoxin levels measured simultaneously

METHODS

The digoxin levels of both serum and urine were determined by the radio-immunological method of Smith et al. (15) A venous blood sample of 0.5 ml was withdrawn and the determination of digoxin was performed in duplicates from 0.1 ml of serum. The antiserum was produced by the method of Smith et al. (16). Rabbits were immunized with a digoxin HSA conjugate

For the determination of the digoxin level in the urine an extraction was performed twice with dichloromethane. The extract was evaporized to dryness and dissolved in a suitable amount of a phosphate buffer solution In this solution the radioimmunoassay was carried out in the same way as in the serum. The average recovery of the method was 89 per cent.

The digoxin clearances were calculated by analyzing the 24 hour urine concentrations and the steady state serum digoxin concentrations on the same day The digoxin clearances were expressed in millilitres per minute per 1 73 square metres of body surface area.

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The daily digoxin dose per kilogram of body weight in these small children was twice as high as that used in adults. The steady state level of serum digoxin corresponded approximately to that of adults. A few fairly high digoxin levels were however measured in infants without any signs of digitalis toxicity

The daily excretion of digoxin in the urine during maintenance therapy was approximately the same in all age groups and on an average 47 per cent of the daily dose This percentage is somewhat lower than that found in adults. The low renal excretion of digoxin in one newborn caused high serum digoxin levels.

The higher dosage requirement of digoxin per kilogram of body weight in children as compared to adults cannot be explained with differences in the renal excretion of digoxin.

KEY WORDS: Neonates, small children, serum digoxin, renal excretion of digoxin

It is well-known that in the treatment of cardiac insufficiency in children a higher dose of digitalis glycosides is needed per kilogram of body weight than in adults In a recent study (8) we showed that to achieve the same serum digoxin level as in adults a dose twice or three times as high per kilogram of body weight is needed for children The reason for this is not known In 1972 Krasula et al (12) suggested a possibility that infants have a more rapid renal excretion of digoxin than older children and adults We have therefore studied the excretion of digoxin in children with congenital heart disease in the steady state of the treatment and compared it with the serum digoxin levels measured simultaneously

METHODS

The digoxin levels of both serum and urine were determined by the radio-immunological method of Smith et al. (15) A venous blood sample of 0.5 ml was withdrawn and the determination of digoxin was performed in duplicates from 0.1 ml of serum. The antiserum was produced by the method of Smith et al. (16). Rabbits were immunized with a digoxin HSA conjugate

For the determination of the digoxin level in the urine an extraction was performed twice with dichloromethane. The extract was evaporized to dryness and dissolved in a suitable amount of a phosphate buffer solution. In this solution the radioimmunoassay was carried out in the same way as in the serum. The average recovery of the method was 89 per cent.

The digoxin clearances were calculated by analysing the 24 hour urine concentrations and the steady state serum digoxin concentrations on the same day The digoxin clearances were expressed in millilitres per minute per 1 73 square metres of body surface area.

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SERUM LEVELS AND RENAL EXCRETION OF DIGOXIN DURING MAINTENANCE THERAPY IN CHILDREN

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ABSTRACT Iisalo, E. and Dahl, M. (Departments of Pharmacology and Paediatrics, University of Turku, Turku, Finland). Serum Levels and Renal Excretion of Digoxin during Maintenance Therapy in Children. Acta Paediatr Scand, 63: 699 1974.—To exclude an increased excretion of digoxin as a possible reason for higher dose requirement in children radioimmunoassay of digoxin in the serum and the 24 hr urine was carried out in 26 children, mainly infants, with congenital heart disease. This procedure was repeated on two consecutive days, in total 64 times during digoxin maintenance therapy

The daily digoxin dose per kilogram of body weight in these small children was twice as high as that used in adults. The steady state level of serum digoxin corresponded approximately to that of adults. A few fairly high digoxin levels were, however measured in infants without any signs of digitalis toxicity

The daily excretion of digoxin in the urine during maintenance therapy was approximately the same in all age groups and on an average 47 per cent of the daily dose. This percentage is somewhat lower than that found in adults. The low renal excretion of digoxin in one newborn caused high serum digoxin levels.

The higher dosage requirement of digoxin per kilogram of body weight in children as compared to adults cannot be explained with differences in the renal excretion of digoxin.

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It is well-known that in the treatment of cardiac insufficiency in children a higher dose of digitalis glycosides is needed per kilogram of body weight than in adults In a recent study (8) we showed that to achieve the same serum digoxin level as in adults a dose twice or three times as high per kilogram of body weight is needed for children. The reason for this is not known In 1972 Krasula et al (12) suggested a possibility that infants have a more rapid renal excretion of digoxin than older children and adults We have therefore studied the excretion of digoxin in children with congenital heart disease in the steady state of the treatment and compared it with the serum digoxin levels measured simultaneously

METHODS

The digoxin levels of both serum and urine were determined by the radio-immunological method of Smith et al. (15) A venous blood sample of 0.5 ml was withdrawn and the determination of digoxin was performed as duplicates from 0.1 ml of serum The antiserum was produced by the method of Smith et al. (16). Rabbits were immunized with a digoxin HSA conjugate

For the determination of the digoxin level in the urine an extraction was performed twice with dichloromethane. The extract was evaporated to dryness and dissolved in a suitable amount of a phosphate buffer solution. In this solution the radioimmunoassay was carried out in the same way as in the serum. The average recovery of the method was 89 per cent.

The digoxin clearances were calculated by analysing the 24 hour urine concentrations and the steady state serum digoxin concentrations on the same day The digoxin clearances were expressed in millilitres per minute per 1 73 square metres of body surface area.

MATERIAL

The material consists of 26 children treated for congenital heart disease in the Paediatric Department University of Turku The age range was from 12 days to 7 years The number of boys was 22 and that of girls 4 The age distribution is seen in Table 1 All patients had a manifest cardiac insufficiency treated with oral digoxin (Lanoxin paediatric Elixir® Well come) usually twice a day On the days of examination the patients had no severe decompensation Diuretics were however given to many patients No one of the patients was treated with spironolactone The congenital heart diseases treated were ventricular septal defect (7 cases) hypoplastic left heart syndrome (3 cases) coarctation of aorta (4 cases) endocardial fibroelastosis (? cases) transposition of the great arteries with ventricular septal defect (? cases) mitral regurgitation (1 case) ventricular septal defect with patent ductus arteriosus (1 case) third-degree atrioventricular block associated with patent ductus arteriosus and aortic stenosis (1 case) tricuspid regurgitation associated with transposition of the great arteries (1 case) tetralogy of Fallot after Waterston operation (1 case) pulmonic atresia with intact ventricular septum (1 case) atrial septal defect (1 case) and cardiomyopathy (1 case) Twelve of the patients had been operated At the time of examination the patients had had digoxin maintenance therapy at least one week After an initial loading dose the treatment was continued with a maintenance dose from 8 to 18 μg per kg of body weight (mean 10)

Blood samples for the determination of serum digoxin were taken on two consecutive days in the morning at least 1? hours after the previous digoxin dose The collection of the daily urine for the determination of digoxin was carried out on the same days In some patients the examination was repeated in the same way several times during infancy Thus the total number of days of successful urine collection was 64 Special care was taken for the 24-hour urine collections It was collected in plastic bags fixed on the skin Whenever there was a doubt of some urine being lost the sample was rejected On the days of examination an ECG was taken and a creatinine or urea nitrogen determination was carried out in addition to the clinical examination No one of the children had signs of renal failure All patients were normokalemic euthyroid and were judged to be adequately digitalized

RESULTS

When studying the clinical condition and the ECG of the patients there were no signs of digitalis toxicity at the time of sampling in spite of some relatively high serum digoxin levels The serum creatinine and urea nitrogen levels were also normal in all patients

The digoxin levels and the urinary excretion of digoxin in different age groups are presented in Table 1 The average excretion of digoxin in the urine increased according to age corresponding to the increased dose When the average daily excretion of digoxin in the urine is calculated as a percentage of the daily dose no significant differences between various age groups can be found In the total material it is 47% of the daily dose

The daily excretion of digoxin in the urine as a percentage of the daily dose in individual patients is shown in Fig. 1 There are some cases in which the excreted amount of digoxin exceeded 100% of the oral daily dose Even a quite low percentual excretion of digoxin was found in some patients There was no statistical evidence for a decreased renal excretion of digoxin in the older children The calculated daily renal clearance of digoxin increased according to age (Table 1)

In one boy with complete transposition of the great arteries and ventricular septal defect the serum digoxin level and the daily renal excretion of digoxin were determined several times (Fig 2) In spite of the normal urine volume this patient excreted extremely little of digoxin (8–13% of the daily dose) as a newborn At the same time the serum digoxin levels of this neonate were high (4 0–2 3 ng/ml) Later the renal excretion of digoxin became normal

DISCUSSION

In this study the average digoxin dose of the children in maintenance therapy was c 10 μg/kg This is lower than the dose of 14 2 μg/kg presented in our earlier study (8) but still about twice as high as the normal dose for adults in the same material The individual variation of serum digoxin levels was at least as great as in adults (e g 11 15) The higher average levels of serum digoxin in newborns and infants from 1 to 3

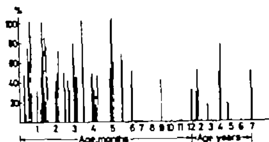


Fig 1 Daily excretion of digoxin in the urine as a percentage of the daily dose in individual patients.

months support the earlier view that in new borns a slightly smaller dose per kilogram of body weight can be recommended

It has been found earlier that the serum half-time of intravenously injected tritiated digoxin is quite similar in adults and children (3). In our study the serum digoxin level corresponds to the average levels in adults (11 15) and children (1 8 14) In some children however high serum digoxin levels were found without any signs of digitalis toxicity This is in agreement with our earlier results (8) and those of Rogers et al (14)

The great care in the collection of urine and on the other hand the correspondence of the urine amounts on the consecutive days support the reliability of the values In the same patient the excretion of digoxin in the urine on two consecutive days did not differ significantly which also supports the reliability of the urine collection

The average renal excretion of the daily digoxin dose during maintenance therapy was 47% Individual differences in the percentage of excretion were striking, however Hernandez et al (6) observed 22% of the radioactively labelled daily digoxin dose in the urine of children with congenital heart disease The condition

Table 1 *Serum and urinary digoxin and renal clearance of digoxin in different age groups*

The real number of patients was 24 but three of the children are included in two different age groups

Age group	Number of patients and (number of investigation days)	Daily dose of digoxin μg/day	μg/kg	μg/m²	Serum digoxin	Excretion of digoxin in the urine (μg/day)	Average daily excretion of digoxin in the urine as per cent of daily dose	Renal clearance of digoxin (ml/min/1 73 m²)
0–30 days	4							
Mean	(8)	30	10	142.5	2.11	14 9	53	53
±S E M					0 34	3.2	12.3	12.5
Range					1 4–4 0	4.5–30 0	102	5–100
1 3 months	9							
Mean	(26)	32	10	141 3	1.6	16.9	55	69
±S.E.M					0.22	1 9	5	11.6
Range					0 2–4.2	7 3–49 7	18.1 102	29–319 0
4–6 month	5							
Mean	(16)	57	11	199	1 1	27 4	51	87
±S E M					0.1	3 0	6	7
Range					0 4–1.5	6 2–51.8	15–103	45–147
7–12 months	1							
	(1)	80	11	216	0.8	33 9	42	122
Over 1 year	7							
Mean	(13)	132	9	201.9	1 4	44 9	36	106
±S E M					0.32	8 4	4.2	23
Range					0.5–4 4	13.5–115.6	13 4–77	21–281
Total materia	26							
Mean	(64)	66.2	10.1	180	1 4	28.3	47	87
±S E.M.		18.8	0.4	15 9	0.22	6.1	3.5	12.3

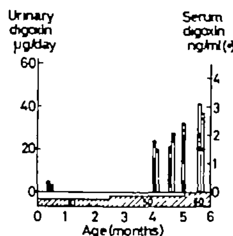


Fig 2 Serum digoxin level and daily urinary excretion of digoxin as compared with daily maintenance dose in a boy with complete transposition of the great arteries and ventricular septal defect

of their patients was however very poor which may explain the lower excretion It is also possible that all of their patients were not yet in the steady state at the time of examination and thus the balance between the tissues and the extracellular fluid had not been reached On the other hand it is possible that some of digoxin metabolites cross-react with digoxin in radioimmunoassay This might cause the higher percentage of excretion found by us However no metabolites of digoxin were found in the urine in the study of Hernandez et al (6)

Krasula et al (12) recently suggested a more rapid excretion of digoxin in the urine in younger children as compared to older ones and the adults Their suggestion was based on the lesser rates of fall in the post absorptive serum digoxin levels with increasing age In our study the excretion of digoxin was not faster in younger age groups

In the group of the neonates one infant out of three had a very low renal excretion of digoxin (Fig 2) The low excretion coincided with a high serum digoxin level This may be due to undeveloped renal function and explains the small dose serum ratio in the group of newborns observed in our earlier study as compared to the older age groups However two out of three newborns has as high renal digoxin excretion as the children in older age groups Individual differences in the speed of renal development can thus be noticed

All examined children were given digoxin in solution (elixir) Recently Huffman & Azarnoff (7) showed in healthy adults that digoxin in solution is absorbed completely whereas the absorption of digoxin in tablets is only 75% Hernandez et al (6) did not find any differences in the digoxin levels excreted in the urine in children after administration of an oral solution intra muscular or intravenous digoxin Also this suggests a complete absorption of orally given digoxin solution The higher postabsorptive serum digoxin levels in younger children found by Krasula et al (12) might be partly due to the form in which the drug was given The older children received digoxin as tablets

In our department we recently studied how different commercial tablet preparations are absorbed and excreted in the urine in the steady state in old people (10) When the best absorbed tablets were used 60% of the daily digoxin dose was excreted in the urine in old people The corresponding mean percentage was 47 of the daily dose in the total material of children If in children digoxin in solution is absorbed completely as in adults the digoxin excretion in the urine in children is even lower than in adults Increased renal excretion of digoxin cannot therefore be considered a reason for the need of a larger dose per kilogram in children as compared with adults This observation of ours is in conformity with the studies by Hernandez et al (6) with radiactively labelled digoxin

The calculation of renal clearance on the basis of the steady state serum digoxin level and the digoxin level of the 24-hour urine differs from the usual clearance calculation Temporary changes in the serum digoxin level caused by the daily digoxin doses tended to increase the clearance values calculated on the

state concentrations The reliability of the 24-hour urine collection has been discussed above. It is natural that if the urine is collected by catheter the determination of the 24-hour renal excretion is more reliable For ethical reasons however we did not consider the use of a catheter justified in infants for research purposes In spite of this inaccuracy our means of renal digoxin clearance were surprisingly close to the values calculated in adults on the basis of the 24-hour urine collected by catheter and to the clearance values measured in healthy adults when radioactively labelled digoxin was administered (3 10) There are no earlier reports of digoxin clearance in children. Digoxin clearance does not seem to be higher in children than in adults in general

In different age groups children seem to have a tendency to an increased level of digoxin clearance from the age of newborn onwards. The digoxin clearance values published earlier in adults are reached as early as the age groups of 4 to 6 months The small number of older children in our material limits our possibility to draw further conclusions

Our study has not explained why children need a higher dose per kilogram of body weight than adults to achieve the same serum digoxin level Many possible assumptions remain unanswered. A different distribution of digoxin is one possibility The relatively higher volume of extracellular fluid in children may cause a lowering of the serum digoxin level On the other hand children may have a different tissue fixation of digoxin This explanation is in contradiction to our observation in one child at autopsy In this case the ratio of digoxin dose per kilogram and the digoxin concentration measured in the heart and the kidney was the same as in digitalized adults at autopsy (9) The distribution of radioactively labelled digoxin in tissues was the same in children as in adults (3 6)

The possibility of a lower binding of digoxin with serum proteins in children is not probable because the protein binding of digoxin seems to be minimal (2)

There were no cases of diarrhoea or vomiting. Malabsorption has been shown to decrease the serum digoxin concentrations (5) Severe malabsorption was not found in our material

The aetiology of heart failure in children and adults is very different and this may in some way influence the sensitivity of the myocardium to digitalis preparations and the requirement of digoxin The individual variation both in the serum levels and the renal excretion of digoxin is at least as great as in adults which emphasizes the individualizing of the digoxin dosage

The determination of digoxin levels has proved valuable in the treatment of children with cardiac insufficiency In the dosing of digoxin the age size and clinical picture of the patient are still the most important criteria but on the basis of digoxin levels it is much safer to change the individual dosis according to the clinical picture Increased digoxin levels do not always cause toxicity in children

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CONCENTRATIONS OF DIGOXIN IN PLASMA AND URINE IN NEONATES INFANTS AND CHILDREN WITH HEART DISEASE

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ABSTRACT Wettrell, G., Andersson, K.-E., Bertler Å. and Lundström, N R. (Departments of Paediatrics and Clinical Pharmacology University Hospital, Lund, Sweden). Concentrations of digoxin in plasma and urine in neonates, infants, and children with heart disease. Acta Paediatr Scand, 63: 705, 1974.—By means of radioimmunoassay and ^{86}Rb-uptake inhibition assay concentrations of digoxin in plasma and urine have been determined in different paediatric age groups. On equal daily maintenance doses (0.012–0.013 mg digoxin/kg b.w./day) higher mean plasma digoxin level was found in full term neonates (3–30 days), 2.1 ng/ml, than in infants (1–12 months) and children (1–10 years), 1.2 and 1.4 ng/ml, respectively On a maintenance dose of 0.019 mg/kg b.w./day one group of infants had an average plasma digoxin level of 2.1 ng/ml (range 1.1–2.9 ng/ml). No signs of toxicity were found. A gradual increase in the renal clearance of digoxin during the first few months of life was demonstrated. There was a highly significant correlation between the clearances of digoxin and creatinine ($r=0.87$ $p<0.001$). It is concluded that the high mean plasma digoxin level in full-term neonates could be explained by low renal elimination of the glycoside

KEY WORDS: Digoxin concentration, renal clearance, radioimmunoassay ^{86}Rubidium method, infants, children

The commonly recommended dose schedules for digoxin treatment of paediatric patients are based on clinical experience Infants and children tolerate higher doses of the glycoside expressed in mg per kilogram body weight (mg/kg b w) than do neonates and adults but clinical and electrocardiographical signs of digitalis toxicity in paediatric patients are often difficult to evaluate (15 18). Since methods for assay of digoxin in plasma became available some studies on plasma digoxin concentrations in infants and children have been reported (5 11 12, 16 17) However the therapeutic concentration range for different age groups has hitherto not been established.

The present investigation is an attempt to further elucidate some of the factors that might affect the relationship between digoxin dosage and plasma glycoside concentration during maintenance therapy in different paediatric age groups Special attention has been given to the renal elimination of digoxin during the first 6 months of life

MATERIAL AND METHODS

Patients

Fifty-three paediatric patients, aged 3 days to 10 years, divided into 3 groups according to age 3–30 days (neonates, $n=12$), 1–12 months (infants, $n=24$), and 1–10 years (children, $n=17$) were studied. Digitalization was undertaken because of congestive heart failure secondary to a wide spectrum of congenital heart defects or paroxysmal tachycardia of supraventricular origin.

According to our prevailing oral dose schedules for digoxin (Lanoxin paediatric solution, B W Ltd, United

Table 1 *Plasma concentrations of digoxin in patients with heart disease*

	Number of patients/number of determinations	*Digoxin dosage* (mg/kg/day)	*Digoxin dosage* ($mg/m^2/day$)	*Plasma digoxin* (ng/ml)
Neonates 0–1 month	12/14	0.013±0.002	0.20±0.03	2.1±0.5 (1.2–3.1)
Infants 1–12 months				
Low dose	10/10	0.012±0.001	0.21±0.03	1.2±0.5 (0.4–1.9)
High dose	14/26	0.019±0.003	0.31±0.04	2.1±0.6 (1.1–2.9)
Children 1–10 years	17/22	0.012±0.002	0.28±0.06	1.4±0.4 (0.6–2.4)
Adults	25/25	0.005±0.001	0.19±0.04	1.2±0.3 (0.7–1.9)

Mean ± standard deviation figures in parentheses represent ranges.

Kingdom) neonates were given 0.05 mg digoxin/kg b.w. as an initial loading dose. Infants were given 0.07 and children 0.05 mg/kg b.w. respectively divided into three doses during 24 h. The subsequent maintenance dose 1/4 of the initial loading dose was then given in two equal amounts daily at an interval of 12 h.

In some infants, a sufficient therapeutic effect was obtained on unchanged daily digoxin dose (mg/day). In these cases because of growth and weight gain the digoxin dose calculated as mg/kg b.w. became lower than the recommended dose. On the other hand in a few infants the maintenance dose was increased because of insufficient therapeutic effect. On basis of these differences in dosage the infant group could be divided into two subgroups (Table 1): low dose infants (0.012 mg digoxin/kg b.w./day) and high dose infants (0.019 mg digoxin/kg b.w./day).

In all patients blood urea nitrogen concentration was within normal limits with respect to age. Serum potassium and acid–base estimates were within normal range. No changes in cardiac action indicating digoxin toxicity were noted in repeated electrocardiograms.

Twenty-five adult patients, controlled at the Departments of Thoracic surgery and Cardiology, University of Lund were included in the study. Their age varied between 43 and 86 years. All were on maintenance treatment with digoxin (Lanacrist Draco Ltd. Sweden) once daily orally the dose varying between 0.25 and 0.50 mg. None of them showed clinical or electrocardiographical signs of digitalis toxicity.

Procedures

After at least 3 days treatment with digoxin in unchanged dose blood samples were collected in the paediatric patients by venepuncture. Samples were taken 10 to 12 h after administration of the previous dose. In the adult patients venous blood samples were drawn 20 to 24 h after the previous digoxin intake.

Plasma digoxin analyses were performed by means of a radioimmunoassay allowing the use of 0.1 ml aliquots of plasma (19). A commercial digoxin kit (Schwarz/Mann) was used, and each sample was analysed in duplicate.

Digoxin concentrations in urine were determined by a modified ^{86}Rb-uptake inhibition assay (2) and also by radioimmunoassay. Urine from non-digitalized, healthy individuals diluted 1:4 with distilled water was used as a blank. To a 100 µl sample of patient urine 2 ml of the blank, 0.5 ml distilled water and 5 ml dichlormethane were added. After shaking, the sample was centrifuged. Samples of 4 and 0.2 ml of the dichlormethane extract were taken and evaporated to dryness in a fume cupboard the evaporates then being dissolved in 1 ml NaCl and 0.6 ml phosphate buffer respectively. The former sample was analysed for digoxin content as described for the plasma ^{86}Rb assay (2) and the latter by radioimmunoassay. The results obtained by the different methods were compared. No significant difference was found. In these experiments, a recovery 103±3.5% (mean+S.E.) of added digoxin (4 ng/ml) was found.

Creatinine in plasma and urine was analysed by means of a picric acid method using a Technicon Autoanalyzer.

Calculations

When calculating digoxin dosage body surface area was determined by means of a nomogram obtained from Crawford et al. (4). Urine was collected for 24 h for determinations of renal clearances of digoxin and creatinine. Daily clearance (=C) was calculated according to the formula

$C_A = U \times V/P$

where U_A=urine concentration of substance A, V= total urine volume, P_A=plasma concentration of substance A. Body surface area used for determinations of clearance values (ml/min/1.73 m^2) for creatinine and digoxin was obtained by means of the formula of Du Bois & Du Bois (6). Statistical significance of paired comparison and of difference between two sample means was evaluated by Student's *t*-test.

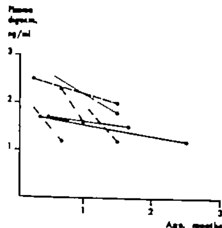


Fig. 1. Plasma digoxin concentration in 7 patients on unchanged maintenance dosage (mg/kg b.w.) during the first 3 months of life. Note decreasing plasma digoxin level with increasing postnatal age.

RESULTS

Plasma digoxin concentrations

The plasma digoxin concentrations in neonates, infants and children are listed in Table 1. For comparison the values in the adult reference group are also given. On comparable maintenance doses calculated on weight basis (0.012–0.013 mg digoxin/kg b.w./day) the mean values (±S.D.) of the plasma digoxin concentrations in "low dose infants" and children were 1.2±0.5 ng/ml and 1.4±0.4 ng/ml respectively. The difference between these values is not statistically significant. These mean values should be compared with that obtained in neonates, 2.1±0.5 ng/ml, given the same daily dose of the glycoside calculated on weight basis. The differences in plasma digoxin concentrations between "low dose infants" and neonates, and between children and neonates are both statistically significant ($p<0.001$).

The plasma digoxin level of adult patients given 0.005 mg digoxin/kg b.w./day (1.2±0.3 ng/ml) did not differ significantly from the values found in "low dose infants" and children although the adult dose of digoxin expressed in mg/kg b.w./day was only about half that used in the paediatric age groups. When digoxin dosage was expressed in square meter body surface area (mg/m²/day) there were no great differences between the doses administered to "low dose infants", children and adults.

Among the infants the plasma levels of digoxin were found to increase from 1.2±0.5 ng/ml in the "low dose" group to 2.1±0.6 ng/ml in the "high dose" group. In 7 patients on unchanged digoxin dose calculated on weight basis plasma glycoside concentrations were measured in the neonatal period and then during the first 3 months of life. The results are illustrated in Fig. 1. As can be seen, there is a decrease in the plasma digoxin level with increasing age in all cases.

Renal clearance of digoxin

The renal clearances of digoxin and creatinine were determined in 15 neonates and infants during the first 6 months of life. Table 2 shows the clinical data and the clearance values of digoxin and creatinine found in these patients. As can be seen in Fig. 2, the renal clearance of digoxin is low during the first month of life but increases with increasing age. The correlation between digoxin clearance (ml/min/1.73 m²) and age (days) is statistically significant ($r=0.63$, $p<0.05$). In patients 1 and 2 clearance values obtained in the neonatal period can be compared with those found a few months later (Table 2).

The relationship between renal clearances of digoxin and endogenous creatinine is shown in Fig. 3. There is a statistically highly significant correlation between these two parameters ($r=0.87$, $p<0.001$). Table 2 gives the amounts of digoxin excreted in the urine during 24 h, expressed as a percentage of the daily maintenance dose.

DISCUSSION

In the present study full-term neonates, "low dose infants" and children were

Table 1 *Plasma concentrations of digoxin in patients with heart disease*

	Number of patients/number of determinations	Digoxin dosage (mg/kg/day)	Digoxin dosage (mg/m²/day)	Plasma digoxin (ng/ml)
Neonates 0–1 month	12/14	0.013±0.002	0.20±0.03	2.1±0.5 (1.[illegible]–3.1)
Infants 1–12 months				
Low dose	10/10	0.012±0.001	0.21±0.03	1.2±0.5 (0.4–1.9)
High dose	14/26	0.019±0.003	0.31±0.04	2.1±0.6 (1.1–2.9)
Children 1–10 years	17/22	0.012±0.002	0.28±0.06	1.4±0.4 (0.6–2.4)
Adults	25/25	0.005±0.001	0.19±0.04	1.2±0.3 (0.7–1.9)

Mean ± standard deviation figures in parentheses represent ranges.

Kingdom) neonates were given 0.05 mg digoxin/kg b.w. as an initial loading dose. Infants were given 0.07 and children 0.05 mg/kg b.w. respectively divided into three doses during 24 h. The subsequent maintenance dose 1/4 of the initial loading dose was then given in two equal amounts daily at an interval of 12 h.

In some infants, a sufficient therapeutic effect was obtained on unchanged daily digoxin dose (mg/day). In these cases because of growth and weight gain the digoxin dose calculated as mg/kg b.w. became lower than the recommended dose. On the other hand in a few infants the maintenance dose was increased because of insufficient therapeutic effect. On basis of these differences in dosage the infant group could be divided into two subgroups (Table 1): "low dose infants" (0.012 mg digoxin/kg b.w./day) and "high dose infants" (0.019 mg digoxin/kg b.w./day).

In all patients blood urea nitrogen concentration was within normal limits with respect to age. Serum potassium and acid–base estimates were within normal range. No changes in cardiac action indicating digoxin toxicity were noted in repeated electrocardiograms.

Twenty five adult patients controlled at the Departments of Thoracic surgery and Cardiology, University of Lund were included in the study. Their age varied between 43 and 86 years. All were on maintenance treatment with digoxin (Lanacrist, Draco Ltd, Sweden) once daily orally, the dose varying between 0.25 and 0.50 mg. None of them showed clinical or electrocardiographical signs of digitalis toxicity.

Procedures

After at least 3 days' treatment with digoxin in unchanged dose blood samples were collected in the paediatric patients by venepuncture. Samples were taken 10 to 12 h after administration of the previous dose. In the adult patients, venous blood samples were drawn 20 to 24 h after the previous digoxin intake.

Plasma digoxin analyses were performed by means of a radioimmunoassay allowing the use of 0.1 ml aliquots of plasma (19). A commercial digoxin kit (Schwarz/Mann) was used and each sample was analysed in duplicate.

Digoxin concentrations in urine were determined by a modified ^{86}Rb-uptake inhibition assay (7) and also by radioimmunoassay. Urine from non-digitalized, healthy individuals diluted 1:4 with distilled water was used as a blank. To a 100 μl sample of patient urine 2 ml of the blank, 0.5 ml distilled water and 5 ml dichlormethane were added. After shaking the sample was centrifuged. Samples of 4 and 0.2 ml of the dichlormethane extract were taken and evaporated to dryness in a fume cupboard, the evaporates then being dissolved in 1 ml NaCl and 0.6 ml phosphate buffer respectively. The former sample was analysed for digoxin content as described for the plasma ^{86}Rb assay (7) and the latter by radioimmunoassay. The results obtained by the different methods were compared. No significant difference was found. In these experiments, a recovery 103±3.5% (mean+S.E.) of added digoxin (4 ng/ml) was found.

Creatinine in plasma and urine was analysed by means of a picric acid method using a Technicon Autoanalyzer.

Calculations

When calculating digoxin dosage body surface area was determined by means of a nomogram obtained from Crawford et al. (4). Urine was collected for 24 h for determinations of renal clearances of digoxin and creatinine. Daily clearance ($=C_A$) was calculated according to the formula

$$C_A = U_A \times V/P$$

where U_A = urine concentration of substance A, V = total urine volume, P = plasma concentration of substance A. Body surface area used for determinations of clearance values (ml/min/1.73 m²) for creatinine and digoxin was obtained by means of the formula of Du Bois & Du Bois (6). Statistical significance of paired comparison and of difference between two sample means was evaluated by Student's t-test.

Acta Pædiatr Scand 63 711–714 1974

LIPIDS IN CORD SERUM AND FREE FATTY ACIDS IN PLASMA IN HEALTHY NEWBORN TERM INFANTS

NIELS CHR. CHRISTENSEN

From the Department of Obstetrics Odense University Hospital Odense Denmark

ABSTRACT Christensen, N Chr (Department of Obstetrics, Odense University Hospital, Odense, Denmark). Lipids in cord serum and free fatty acids in plasma in healthy newborn term infants. Acta Paediatr Scand, 63:711 1974.—Serum cholesterol triglycerides, and glycerol in cord serum and plasma FFA in cord blood and at 1½, 6, 12, 24 and 48 hours after birth were determined in 18 healthy term infants. Concentrations of lipids in cord blood were low; and there were no correlation between cord lipids and subsequent FFA values. A rapid increase in FFA level, with peak values at 12 hours, was seen. Significant, negative correlations were found between FFA concentration and rectal temperature at 1½ hour and between total caloric intake and FFA concentration at 48 hours.

KEY WORDS Free fatty acids, triglycerides, cholesterol, glycerol, newborn infants

During the first days of life before oral caloric intake becomes adequate free fatty acids (FFA) play a key role in the energy metabolism of the infant In normal newborns plasma FFA concentration rises during the first few hours of life indicating lipolysis (17 18) Several factors including starvation epinephrine release and cold stress may enhance lipolysis (22) but conflicting evidence regarding the significance of such variables in the production and regulation of early postnatal lipolysis documents the need for further investigation The present study was undertaken in order to assess plasma FFA courses during the first 48 hours of life in healthy term infants and to learn whether certain variables of cord blood, the rectal temperature and caloric intake influence the FFA level.

MATERIAL

Ten boys and eight girls borne by healthy mothers with regular menstrual cycles, reliable menstrual data, and uneventful pregnancies were studied. The mothers were seen at regular intervals throughout the pregnancy and none had arterial hypertension, glucosuria, or proteinuria. Deliveries were spontaneous None of the infants was asphyxiated at birth (Apgar score >8 at one and five minutes) Birth weights ranged from 3150 to 4100 g. In each case good agreement was found between menstrual data and clinical assessment of gestational age which ranged from 277 to 290 days Following tying of the cord the infants were kept in heated boxes for about one hour and then transferred to ordinary cribs.

METHODS

Immediately following delivery blood was drawn from the umbilical vein, part of the sample being stabilized with EDTA. Samples were centrifuged immediately or within one hour following storage at 4°C. Analyses were performed on fresh centrifuged plasma or following storage at −22°C. Capillary samples, obtained from a well perfused warm heel, were treated in the same manner

Capillary samples were taken at 1½ hours (85–120 min), 6 hours (305–420 min) 12 hours (600–840 min), 24 hours (21–27 hours) and 48 hours (43–52 hours)

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During the first days of life before oral caloric intake becomes adequate free fatty acids (FFA) play a key role in the energy metabolism of the infant. In normal new borns, plasma FFA concentration rises dur ing the first few hours of life indicating lipolysis (17 18) Several factors including starvation, epinephrine release and cold stress may enhance lipolysis (22) but conflicting evidence regarding the significance of such variables in the production and regu lation of early postnatal lipolysis documents the need for further investigation The present study was undertaken in order to assess plasma FFA courses during the first 48 hours of life in healthy term infants and to learn whether certain variables of cord blood, the rectal temperature and caloric intake influence the FFA level

MATERIAL

Ten boys and eight girls borne by healthy mothers with regular menstrual cycles, reliable menstrual data, and uneventful pregnancies were studied. The mothers were seen at regular intervals throughout the pregnancy and none had arterial hypertension, glucosuria, or proteinuria. Deliveries were spontaneous None of the infants was asphyxiated at birth (Apgar score >8 at one and five minutes) Birth weights ranged from 3 150 to 4 100 g. In each case good agreement was found between menstrual data and clinical assessment of gestational age which ranged from 277 to 290 days Following tying of the cord, the infants were kept in heated boxes for about one hour and then transferred to ordinary cribs

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Table 1 *Mean values standard deviation (S D) and range for plasma FFA and serum cholesterol triglycerides and glycerol in umbilical venous blood*

Values stated are in mM/l Numbers of observations are shown in parentheses

	FFA (18)	Cholesterol (17)	Triglycerides (16)	Glycerol (16)
x̄	0 [illegible]4	[illegible] 3	0 5[illegible]	0 09
S D	0 11	0.5	0 16	0 02
Range	0 11–0 48	1 5–3 1	0 3[illegible]–0 93	0 06–0.14

Laurell & Tibbling (14) modified to allow single determinations to be performed on [illegible]0 μl aliquots. Coefficients of variation for cholesterol and triglyceride analysis were 5% and 3% respectively FFA values stated represent means of two determinations, the standard deviation for single measurements being 0.03 mM/l for the range of values reported. A running palmitic acid standard (0 40 mM/l) was analysed with a coefficient of variation of 7%.

Rectal temperature was obtained at the time of blood sampling and oral feedings were registered.

RESULTS

Data on cord blood are shown in Table 1 Concentrations of the various constituents of the lipid status varied considerably No correlation was found between concentrations of cholesterol and triglycerides triglycerides and glycerol triglycerides and FFA and FFA and glycerol respectively

In 10 infants lipoprotein electrophoresis demonstrated a small amount of β-lipoprotein whereas the presence of a præ β-lipoprotein could not be detected in any case The average concentration of cholesterol in samples containing a β-lipoprotein (2 6 mM/l) was slightly higher than that in samples where β-lipoprotein was not demonstrated (2 0 mM/l)

Plasma FFA concentrations found during the first 48 hours are shown in Table 2 Peak values at 12 hours were significantly higher than values found at 6 and 24 hours and a significant decrease occurred between 24 and 48 hours Statistical analysis failed to disclose any correlation between concentrations of FFA and triglycerides in cord blood and the FFA level at 1½ hour

Mean rectal temperatures at 1½ hours 6 hours 12 hours 24 hours and 48 hours were 35 4°C 36 0°C 36 2°C 36 4°C and 36 4°C respectively The FFA concentration of plasma obtained at 1½ hours but not that of subsequent samples was found to be negatively and significantly correlated to the rectal temperature ($r = 0\ 50$ $p < 0\ 05$) especially in the low temperature range (Fig. 1) However even in infants with rectal temperatures above 35.5°C a considerable postnatal rise in the FFA level could be demonstrated at this point Differences in rectal temperature and FFA values between infants at (appr) 1½ hours were unrelated to the small differences in age (85–120 min)

An attempt was made to evaluate the significance of oral caloric intake for the changing FFA level Volumes of breast milk were estimated by arbitrarily multiplying numbers of breast feedings on the first day by 10 ml and those on the second day by 20 ml The average caloric value of oral fluids given was taken to be 500 Cal/l Mean estimated caloric intake on day one was 35 Cal and on day two 158 Cal For values obtained at 24 hours no correlation between caloric intake and FFA concentration could be established whereas the FFA concentrations at 48 hours were significantly and negatively correlated to the caloric intake during the preceding

Table 2 *Mean values and standard deviation (S.D) for plasma FFA during the first 48 hours of life in healthy term infants*

Numbers of observations are shown in parentheses

	Age (hours)					
	0 (18)	1½ (17)	6 (17)	12 (17)	24 (18)	48 (18)
FFA (mM/l)	0 [illegible]4	0 84	0 77	1 03	0 7[illegible]	0.54
S D	0 11	0 29	0 28	0 35	0.29	0 24
p*		<0.01	>0.05	<0 025	<0.01	<0.05

* Significance of difference between means (t-test).

24-hour period ($r=0.57$ $p<0.05$) as well as to total caloric intake ($r=0.64$ $p<0.01$)

DISCUSSION

The observations of low levels of lipoproteins, cholesterol triglycerides and FFA in cord blood confirm several previous reports. The mean cholesterol concentration observed by Kaplan & Lee (11) 2.38 mM/l (95 mg/100 ml) is slightly higher than the present value whereas somewhat lower cholesterol and triglyceride levels have been reported by Kwiterovich et al (13) Brody & Carlson (2) and Persson & Gentz (18). The FFA values found in the present study are very similar to those reported by Persson & Tunell (19) using the same method. In adults genetic and nutritional factors are known to influence the serum lipid levels. However because the cholesterol concentration of umbilical venous serum seems unrelated to the maternal cholesterol level (15) and the maternal social status (15) as well as to birth weight (21) and sex (13) the relatively high mean cholesterol concentration found in the present investigation does not seem to originate in the selection of mothers and infants for study. Children with familial hypercholesterolemia seem to have elevated umbilical venous cholesterol concentrations (6 13). In such cases genetic factors may be involved. The close agreement between the umbilical FFA level in our study and in that of Persson & Tunell (19) renders differences in sampling technique an unlikely cause of the differences in cholesterol level observed. Probably such differences depend in part on the chemical technique employed. Similar considerations may be applied to the differences in umbilical venous triglyceride concentration reported.

The early postnatal rise in FFA and glycerol (19) reflects significant lipolytic activity during the first hours of life. The mechanism of induction of postnatal lipolysis however is incompletely understood. Van Duyne et al.

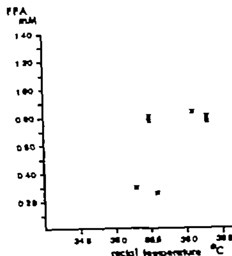


Fig. 1. Relationship of FFA to rectal temperature at 1½ hours after birth. The equation of the regression line is $y=-0.25x+9.68$

(4) found that in the newborn lamb administration of a ganglionic blocking agent (hexamethonium) provoked a fall in plasma FFA concentration to the level of umbilical venous plasma, suggesting that the effect is mediated by the sympathetic nervous system.

Early neonatal cold stress may be an important factor in the production of lipolysis. Studies in newborn rabbits and lambs have shown that animals kept in cool surroundings from the moment of birth develop a significant rise in plasma FFA whereas animals not exposed to cold stress show a much smaller rise (1) or fail to respond, even if feedings are withheld for 24 hours (7). However comparing plasma FFA levels of newborn infants nursed at different ambient temperatures Persson & Tunell (19) were unable to demonstrate any difference. Pribylova & Rylander (20) observed that in infants kept warm from the moment of birth the plasma FFA concentration did not reach the level seen in infants with early cold stress until 140 min after delivery. In the blood of newborn infants Chen et al (3) found a significant correlation between concentrations of glucose and FFA which was not reproduced in the studies by Persson & Tunell (19). By infusion of glucose in the umbilical vein im

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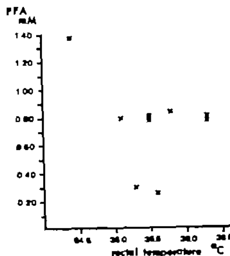


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mediately following delivery followed by frequent oral feedings Novak et al (16) were able to prevent the rise in plasma FFA during the first 12 hours of life in normal infants

The pronounced rise in plasma FFA and the negative correlation of FFA concentration to rectal temperature at 1½ hours found in the present study lend support to the view that cold exposure is a contributory factor in the initial development of postnatal lipolysis Possibly the initial period of relative caloric insufficiency may have contributed to the sustained high levels of FFA present from 12 to 48 hours after birth In studies in which infants were kept fasting for 24 hours after birth plasma FFA values did not decrease significantly from 12 to 24 hours after birth (12 16) It may be assumed therefore that the fall in plasma FFA at this point observed in the present study was related to the oral caloric intake which was negatively correlated to the FFA level at 48 hours

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THE ROLE OF SERUM FACTORS IN THE LYMPHOCYTE TRANSFORMATION TEST OF CHILDREN WITH ACUTE LEUKAEMIA

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ABSTRACT Révész, T., Szigeti, R. and Schuler D. (Second Department of Paediatrics, Semmelweis University Medical School, Budapest, Hungary). The role of serum factors in the lymphocyte transformation test of children with acute leukaemia. Acta Paediatr Scand, 63:715, 1974.—PHA-induced lymphocyte transformation tests were carried out in 14 children who were in full remission of acute lymphoblastic leukaemia and in 12 controls. Stimulation was assessed on the basis of tritiated thymidine uptake. For an adequate testing of lymphocyte reactivity dose–response curves were established for each child. No significant difference could be observed in either the maximum response or the shape of the curve, between the leukaemic and the control groups. Supplementation of the culture medium with autologous plasma instead of AB serum did not result in any significant inhibition or stimulation of lymphocyte reactivity to PHA. The lymphocyte response of both leukaemic and control children, however was greatly reduced when sera from untreated leukaemic children was used. Allogeneic sera drawn from patients in remission exerted little or no inhibitory effect, while that obtained in relapse was again more inhibitory The observed effect was not due to cytotoxic antibodies, nor due to natural iso-antibodies, but is thought to be a phenomenon closely connected with the active phase of malignant disease. The exact nature of the agent is still far from being clarified at present.

KEY WORDS Acute lymphoblastic leukaemia, lymphocyte transformation test, PHA dose–response curve leukaemic serum factors

The Phytohaemagglutinin (PHA) stimulation of blood lymphocytes has been widely used for the assessment of lymphocyte reactivity in a great variety of diseases The test has also been applied to the investigation of children with acute lymphoblastic leukaemia (ALL) and was found to be normal in children who were in full remission of their disease (10) The dose used in these experiments however was thought to be higher than the one giving the most sensitive discrimination between normal and abnormal responses Furthermore the maximum responses of individuals can be quite different for a chosen dose of PHA which makes comparisons rather difficult (5) Recently Lauder & Bone (11) have called attention to the need to determine dose–response curves for PHA when comparing the lymphocyte stimulation of patients with carcinoma, with those of controls

The clinical evaluation of children with ALL and a threefold incidence of negative skin test to the purified protein derivative of

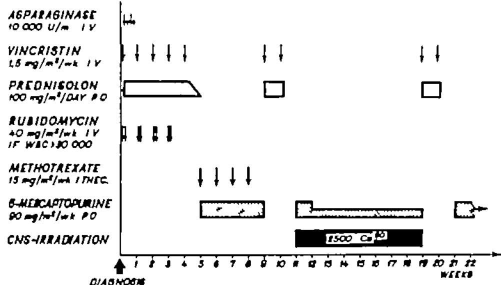


Fig 1 Acute lymphoid leukaemia.—Treatment protocol for details see text.

tuberculin (PPD) as compared with controls[1] aroused our interest in the lymphocyte functions of these patients

The ability of their lymphocytes to transform under the influence of PHA the effect of the patients autologous plasma and untreated leukaemic serum on lymphocyte transformation was investigated

MATERIALS AND METHODS

1 *Leukaemia patients and treatment*

Fourteen children between 2 and 14 years of age with acute lymphoblastic leukaemia were studied. They were all in their first remission state (average remission duration 18 months range 4–62 months) Three of them were off all chemotherapy the rest were receiving maintenance and re-inductions at regular intervals (Fig. 1)

Asparaginase vincristine and prednisolone are administered for the achievement of remission Intrathecal methotrexate and ^{60}Co irradiation are used for the prevention of CNS leukaemia. Maintenance is carried out by using 6-mercaptopurine interrupted by regular 2 monthly re-induction periods Therapy is continued for a total of 3 years and if the child is still in complete remission all drugs are then discontinued.

No cytostatic drugs were given ? days prior to testing. 12 children hospitalized for minor surgical intervention or non-haematological investigations were studied as controls

[1] BCG vaccination of newborn babies is a routine procedure in Hungary a positive skin test to PPD therefore is almost universal.

2 *Lymphocyte cultures*

Some 10–15 ml heparinized blood was mixed with an equal volume of TC 199 and lymphocytes separated by Ficoll gradient centrifugation Cells comprising of 95–96% lymphocytes 1–2% monocytes 2–3% polymorphonuclears were washed three times in TC 199 Conical glass tubes (10×105 mm) and later Falcon-II microculture plates were used for the cell cultures 2×10⁵ lymphocytes were cultured in a final volume of 0 2 ml of TC 199 In order to estimate the lymphocyte reactivity to PHA (Difco PHA-P control 573353) dose–response curves were established for each child tested. The mean of triplicate cultures was counted for each dose (0 01 0.1 1 5 10 50 μg PHA). Pooled AB serum the patients own plasma or leukaemic serum—all in a final concentration of 20%—were used for the supplementation of the culture medium.

Cultures in tubes or Falcon-II plates were incubated for 70 hours in a humid atmosphere containing 5% CO_2. For the estimation of DNA synthesis 1 μCi of tritiated thymidine (^{3}H-thymidine-methyl spec act. 10 Ci/mmol Radiochemical Centre Amersham) was added to each culture followed by a further incubation period of 2 hours Cell suspensions were filtered on Whatman GF/C filter discs washed with 5% trichloroacetic acid and methanol and placed in counting vials The incorporated activity was measured in a Packard tri-carb scintillation counter After correlation for background the results were expressed in counts per minute (cpm) *Statistical analysis* was carried out using Students *t*-test

Sera were kept at −96°C prior to testing and were thawed only once All sera were absorbed with AB Rh_0 (D) positive red blood cells and tested for cytotoxic activity using the standard NIH-microlymphocytotoxicity method

Quantitative immuno-electrophoresis was carried out by using horse antihuman heavy chain antisera (Human, Budapest).

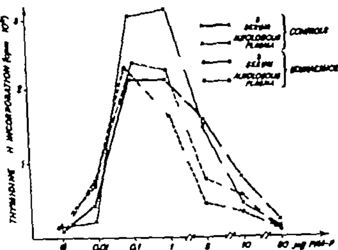


Fig. 2. Phytohaemagglutinin dose-response curves in children with leukaemia in remission and controls.

RESULTS

Maximum response to PHA and the shape of the curve shows no appreciable difference between the leukaemic children in remission and the controls (Fig. 2). Deviations from the mean are great in both groups (Table 1). Parallel tests were run using AB serum or the patients' own plasma. Statistical analysis using Students *t*-test revealed no significant differences between either the leukaemic and control groups or between results obtained with AB serum or autologous plasma.

To test the effect of serum from untreated leukaemic patients blood was drawn from children with acute lymphoblastic leukaemia prior to all treatment. The mean of triplicate cultures using AB serum was taken as 100% in the case of each individual lymphocyte donor and the mean results obtained with other sera were expressed as a percentage of that with AB serum. The effect of sera on PHA reactivity was tested on the lymphocytes of a few patients with acute lymphoid leukaemia in remission and in several controls (Table 2). Untreated leukaemic serum was inhibitory in all cases though to varying degrees on cells from dif-

Sera obtained from children with full remission of ALL showed no or a lesser degree of inhibition on other children's lymphocytes while serum from a patient in relapse had a more inhibitory effect on PHA responsiveness. Deviations from the mean values were considerable with all the sera tested.

None of the sera were found to be cytotoxic. Immuno-electrophoresis revealed no regular change in the sera investigated except for an increased level of α_1-glycoprotein in the truly inhibitory ones. Other sera with an increased level of α_1-glycoprotein obtained from non-leukaemic patients showed no significant inhibition of lymphocyte transformation (Révész, unpublished data).

DISCUSSION

Preliminary tests investigating lymphocyte reactivity in normal children and in children with Down's syndrome showed great variability in the maximum responses to PHA (13). To avoid differences arising from this source dose–response curves were established for each child in these series of experiments. Both the maximum response and the shape of the dose–response curve were practically the same in the leukaemic and

Table 1 *Phytohaemagglutinin dose responses in children with ALL in remission and controls (mean cpm ±1 S D)*

PHA dose (μg/0 ² ml)	Controls (12 cases) AB serum	Controls (12 cases) Autologous plasma	Leukaemics (14 cases) AB serum	Leukaemics (14 cases) Autologous plasma
0	87 (±43)	160 (±142)	118 (±97)	154 (±121)
0 01	439 (±643)	248 (±399)	719 (±647)	755 (±1 069)
	0 4>p>0 3		0 475>p>0 45	
0 1	2 167 (±1 561)	2 955 (±1 759)	2 230 (±2 005)	2 355 (±² 124)
	0 2>p>0.15 (controls vs leukaemics, AB serum)	0 3>p>0 25 (controls vs leukaemics, autologous plasma)		
	0 1>p>0 05		0 3>p>0.25	
1	2 076 (±2 139)	3 146 (±1 974)	1 629 (±1 719)	2 206 (±2 214)
	0 3>p>0 25 (controls vs leukaemics, AB serum)	0 15>p>0 1 (controls vs leukaemics, autologous plasma)		
5	1 568 (±1 789)	1 435 (±368)	454 (±465)	769 (±617)
10	803 (±1 130)	438 (±379)	367 (±359)	471 (±613)
50	234 (±167)	153 (±95)	102 (±74)	120 (±57)

the control group This is in good agreement with previous findings (10 2) and excludes a shift in PHA sensitivity to higher doses as observed in chronic lymphocytic leukaemia

The observed large deviations from the mean on the other hand call attention to the fact that comparative studies using one chosen dose of PHA in small numbers of patients can have very misleading results

Considerable interest has been focused recently on the role of serum factors in the lymphocyte transformation test of patients with acute leukaemia (14 7 8) In our experiments no significant difference could be detected in PHA stimulation when autologous plasma was used instead of AB serum The same phenomenon was observed by Gutterman et al (8)

Lymphocyte transformation from both leukaemic and normal children was greatly reduced however when the culture medium was supplemented with serum drawn from leukaemic patients prior to all therapy The degree of inhibition varied from one lymphocyte donor to another a fact we cannot explain at present As all sera were absorbed with AB Rh (D) positive red blood cells and were not found to contain cytotoxic antibodies the observed differences are due to other factors It is challenging to contemplate that the observed inhibitory phenomenon is analogous to the blocking effect of carcinoma serum on lymphocyte cytotoxicity described by Hellström et al (9) Their sera however were tumour type specific whereas the inhibition observed in our experiments occurred in a highly non specific sys

Table 3 *The effect of leukaemic serum on the lymphocyte transformation induced by PHA*

Source of serum	Transformation: % of mean cpm obtained with AB-serum	Mean (%)	Cyto-toxicity	Immuno-electrophoresis: IgG (mg%)	IgA (mg%)	IgM (mg%)	α_2-glycoprotein
AB (normal)	100			800–1 400	140–240	50–190	
Untreated ALL							
1	0* 0, 1 5 7 8* 10 17* 29 51*, 69*	17 9	–	676	30.4	32.4	Increased
	4, 7 9 40, 65 76 80 93	46 7	–	1 184	144	108	Increased
3	25 53	39 0	–	N T	N T	N T	
ALL in remission							
1	3 32, 43 68 108 147	66.8	–	3 000	12	164	Normal
	78, 96, 99 155 192, 220	140 0	–	700	116	46 8	Normal
ALL in relapse	0, 0 .. 6 16, 21 27 31 53 68, 81 109	34.5	–	600	122	128	Increased

Cell donors leukaemic children in remission
N T = Not tested

tem similar to the findings of Golob et al (6) and Al-Sarraf et al. (1)

Sjögren (12) suggested that the blocking agents might be antigen-antibody complexes which would point to a competing mechanism between these complexes and PHA for receptor sites on the lymphocyte membrane

Cooperband et al (3 4) reported the depression of lymphocyte transformation by an extract of normal human plasma containing the alpha globulin fraction. Immunoelectrophoresis of the tested leukaemic sera in our experiments revealed an increase in the alpha globulin fraction in all the inhibitory sera For this reason we investigated the effect of other sera from non-leukaemic patients where an increase of the alpha globulin fraction was observed but no conclusive results could be obtained The nature of this non-specific inhibitory substance requires further study

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The authors wish to thank Dr Éva Paulik for the immunoelectrophoretic studies, Mrs Éva Gyódi for the lymphocytotoxicity investigations and Miss Andrea Molnár for excellent technical assistance

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3 *The effect of leukaemic serum on the lymphocyte transformation induced by PHA*

	Transformation			Immuno-electrophoresis			
of serum	% of mean cpm, obtained with AB-serum	Mean (%)	Cyto-toxi-city	IgG (mg%)	IgA (mg%)	IgM (mg%)	α_2-glycoprotein
				800–1 400	140– 40	90–190	
rmal)	100						
ted ALL	0* 0, 1 5 7 8* 10 17* 29 51 69*	17 9	-	676	30 4	32 4	Increased
	4 7 9 40, 65 76 80 93	46.7	-	1 184	144	108	Increased
	25 53	39 0	-	N T	N T	N T	
a remission	3 32, 43 68 108 147	66.8	-	3 000	12	164	Normal
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POSTNATAL DEVELOPMENT OF RENAL HYDROGEN ION EXCRETION CAPACITY IN RELATION TO AGE AND PROTEIN INTAKE

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From the Department of Paediatrics University Hospital Lund, Sweden

ABSTRACT Svenningsen, N. W and Lindquist, B. (Department of Paediatrics, University Hospital, Lund Sweden). Postnatal development of renal hydrogen ion excretion capacity in relation to age and protein intake. Acta Paediatr Scand, 63, 721 1974.—The cumulative and maximum renal hydrogen ion excretion capacity after induced acidosis has been studied in six term and twenty preterm infants at 1–3 and 4–6 weeks of postnatal age corresponding to for preterm babies 34–36 and 37–39 and for term babies 41–43 and 44–46 weeks of gestational age. The maximum net hydrogen ion (H_{max}) excretion capacity is lower in preterm than in term infants at 1–3 weeks of postnatal life. However when approaching full gestation there is a considerable increase of the H_{max} excretion capacity of preterm infants almost equal to that found in term infants at 1–3 weeks of age. High dietary protein intake lasting for in average two to three weeks in preterm infants does not change the maximum renal H excretion during induced acidosis, but significantly enhances the initial increment of excretion rate of titratable acid (H_{TA}) in comparison to preterm infants fed low protein diets. The findings of the present investigation suggest that the development of renal hydrogen ion excretion capacity in preterm infants during the first weeks of life is related primarily to gestational age. This development may however to certain degree respond to increased excretory needs imposed by dietary load.

KEY WORDS: Neonates, renal hydrogen ion excretion, postnatal development, dietary composition

In studies concerning the postnatal development of kidney functions an important question is whether a high protein or solute stress in early life stimulates the postnatal growth of the kidney and thereby also the development of renal glomerular and/or tubular functions Some animal experiments and some clinical reports (3 6 12, 13 22) would seem to favour such a hypothesis Certain investigations have pointed out the risks from overloading less well developed renal excretory functions in early life (2 14 15 28). Yet longitudinal studies with respect to this problem have not been reported

In previous investigations we have studied the incidence of metabolic acidosis in relation to protein intake in early infancy (26) as well as the development of renal acid-base regulating mechanisms by titration studies at this age (27) The aim of the present investigation has been to evaluate the postnatal development of the renal acidification capacity by measuring the renal hydrogen ion excretion before and after induced acidosis (1) in relation to gestational and postnatal age and (2) in relation to the protein intake in preterm infants

MATERIAL AND METHODS

Clinical material

Twenty-six infants were studied between 1 and 6 weeks of postnatal age. The study comprised 6 term AGA

Table 1 *Clinical data Birth weight gestational age at birth days of age at study procedures and type of formula in the different experimental groups*

	Birth weight (g)	Gestational age (weeks)	Age at study (days)		Formula (see Table 2)
			1st	2nd	
Term AGA infants					
Group I ($N=6$)					A
Mean	3 340	39	17	36	
Range	2 950–3 800	38–40	14–21	30–4	
Preterm AGA infants					
Group II A ($N=6$)					A
Mean	1 925	32	18	39	
Range	1 300–2 300	31–35	14– 1	35–41	
Group II B ($N=7$)					B
Mean	2 070	33	16	34	
Range	1 790–2 430	32–35	11–21	28–40	
Group II C ($N=7$)					C
Mean	2 125	33	19	38	
Range	1 760–2 470	31–36	15–21	32–42	

(appropriate-for-gestational age) and 20 preterm AGA infants. Infants with an abnormal perinatal period e g severe asphyxia or respiratory distress syndrome were excluded Parental and institutional consent had been obtained in all cases

All infants were studied at two occasions. At the first study the postnatal age was 1–3 weeks and at the second one 4–6 weeks This corresponds to 34–36 and 37–39 gestational weeks respectively for preterm infants 41–43 and 44–46 gestational weeks respectively for term infants

The clinical data of the infants are presented in Table 1 The infants were divided in two groups Group I= term AGA infants and Group II=preterm AGA infants The term infants were all fed a humanised formula (formula A) The preterm infants were divided in three subgroups (II A II B and II C) according to the dietary protein intake (formula A B and C) The mean birth weight and gestational age of the subgroups did not differ significantly The degree of maturity of each baby was estimated as previously described (6)

The dietary composition of formulas A B and C are presented in Table 2 The daily amount of formula supplied was equal in both groups i e 140 to 150 ml/kg/day The protein content of the formulas was 2 3 g/100 kcal, 3 3 g/100 kcal and 5 8 g/100 kcal or expressed as percentage of calories as protein 9% 15% 24% Thus the daily amount of protein intake attained was 2.4 g/kg/day (formula A) 3 3 g/kg/day (formula B) and 5 7 g/kg/day (formula C) respectively In each subgroup of preterm infants The daily dietary solute load was 19 4 mOsm/kg/day 26 8 mOsm/kg/day and 49 1 mOsm/kg/day with formulas A B and C respectively From day 6 after birth either formulas A B or C was given during the entire study period.

Study procedure

Single dose short term NH_4Cl loading tests were performed in each infant at 1–3 and 4–6 weeks of postnatal age (Table 1) The dosage was 100 mEq per m^2 body surface Urine specimens were collected 4 hours before (preloading control) and one to two-hourly for 8 hours following the NH_4Cl load as has been described in detail in a previous report (27) In addition in the infants fed formula A (group I and II A) 8-hourly urine specimens were obtained on both days prior to the NH_4Cl load. Capillary blood acid-base status was determined before and every second hour after the NH_4Cl load

Laboratory analyses

The methods for determination of capillary blood acid-base status and for urinary titrations of net acid (H_{NAE}) titratable acid (H_{TA}) ammonia (H_{NH_4}) and net base excretion as urinary bicarbonate ($HCO_{3\,NB}$) have been described earlier (4 17 26 27)

Statistical methods

Comparison between the different groups with regard to blood acid-base data and urinary H -excretion has been made by means of Student s *t*-test using the following scale of statistical difference

$0.05 < p$	not significant	n s
$0.01 < p < 0.05$	possibly significant	
$0.001 < p < 0.01$	significant	
$p < 0.001$	highly significant	

RESULTS

A *Maximum Renal H^+ Excretion after Induced Acidosis*

1 *Re gestational and postnatal age*

Fig 1 shows the plasma base deficit values and the values of urinary H^+_{NAE}-excretion 2 days before and on the day of NH_4Cl

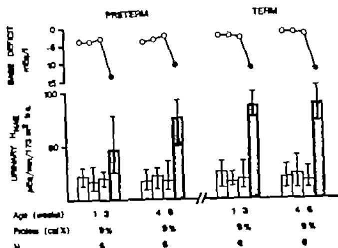


Fig. 1. Influence of age on plasma base deficit and urinary H^+_{NAE} excretion before and during induced acidosis. Preterm and term infants at 1–3 and 4–6 weeks of postnatal life, i.e. 34–36, 37–39, 41–43, 44–46 weeks of gestational age. Symbols: Preloading and maximum plasma base deficit (mean) ○—● and urinary H_{NAE} (mean and range) [bars]

Table 2. *Composition of formulas*

	Type of formula		
	A	B	C
Major constituents			
Protein (g/100 kcal)	2.3	3.3	5.8
Protein (g/l)	16	22	38
Fat (g/l)	35	4	30
Carbohydrate (g/l)	72	86	58
Minerals (g/l)	1.7	2.6	4.2
Energy (kcal/l)	685	665	650
% of calories as			
Protein	9%	15%	4%
Fat	46%	34%	41%
Carbohydrate	45%	51%	35%
% of protein as			
Casein	40	80	80
Whey	60	20	20
Major components of minerals			
Sodium (mEq/l)	9	13	17
Potassium (mEq/l)	17	25	37
Chloride (mEq/l)	13	15	18
Calcium (mg/l)	350	700	1 200
Phosphorus (mg/l)	300	400	950
Iron (mg/l)	14	10	10
Magnesium (mg/l)	40	55	120
Total solutes[a] (mOsm/l)	129.4	178.8	327.7
Formula acid and base content			
FNBC[b] (mEq/l)	15	33	50
TA_{nf} (mEq/l)	2.4	5.9	9.8

[a] Total dietary solute content as calculated according to Fomon (10) assuming that all dietary protein is excreted as urea (1 g of dietary protein yields approximately 5.7 mOsm of urea) and that all sodium, chloride, potassium and 10% of phosphorus of the diet is excreted. The contribution of calcium to renal solute load is minimal and can be ignored.

[b] Formula net base content = $(Na^+ + K^+ + Ca^{++} + Mg^{++}) - (Cl^- + 1.8 \cdot P)$.

Direct titratable acidity of the formula.

loading test in 6 preterm (subgroup II A) and 6 term (group I) infants at 1–3 and 4–6 weeks of postnatal age corresponding to for preterm babies 34–36 and 37–39 and for term babies 41–43 and 44–46 weeks of gestational age. The protein intake was equal (formula A) and unchanged during the study.

The preloading values of base deficit and urinary H_{NAE} did not differ significantly from day to day. During the NH_4Cl induced acidosis a significant decline of the base deficit was obtained in all infants. The obtained values correspond to plasma bicarbonate values well below the renal bicarbonate threshold level at this age, thereby a maximum challenge on the renal H^+ excretion ability had been attained (5).

In preterm infants there is with increasing age a significant enhancement of the maximum H^+_{NAE}-excretion during induced acidosis (Fig. 1, Tables 3*a* and 3*b*, $p<0.001$). In term infants the H^+_{NAE}-excretion capacity is well developed already at 1–3 weeks of postnatal age (41–43 gestational weeks) being significantly higher than in preterm infants at 4–6 weeks of postnatal age (37–39 gestational weeks) (Fig. 1, Tables 3*a* and 3*b*, $p<0.001$).

2. *Re dietary protein intake*

The response to NH_4Cl induced acidosis was in the preterm babies also studied in

Table 3*a* *Preloading levels and maximum change of blood pH plasma base deficit and urinary pH and H^+ excretion values during NH_4Cl induced acidosis in term and preterm infants at 1–3 weeks of postnatal age Significance of difference comparing group II A with group I II B and II C respectively*

	Gestational age (weeks)	Blood pH		Plasma base deficit (mEq/l)		Urine pH	
		pre	max	pre	max	pre	max
Term infants							
Group I (N=6)	41–43						
Mean		7 39	7 27	−1	−11.8	7.31	4.94**
S E.M		0 01	0 01	0 77	0 36	0.03	0 03
Preterm infants							
Group II A (N=6)	34–36						
Mean		7.35	7 20	−2.3	−13 1	7 15	5 99
S E.M		0 01	0 01	0 61	0 40	0.17	0 01
Group II B (N=7)	34–36						
Mean		7 31	7 19	−5 9	−13 1	7 11	6 00
S E.M		0 01	0 02	1 39	0 61	0 12	0.04
Group II C (N=7)	34–36						
Mean		7 30*	7 18	−7 6	−13.8	6 77	6.04
S E.M		0 01	0 01	1 70	0.57	0 04	0 05

$p<0.05$ $p<0.01$ $p<0.001$ S E.M =Standard error of the mean.

Table 3*b* *Preloading levels and maximum change of blood pH plasma base deficit and urinary pH and H^+ excretion values during NH_4Cl induced acidosis in term and preterm infants at 4–6 weeks of postnatal age Significance of difference comparing group II A with group I II B and II C respectively*

	Gestational age (weeks)	Blood pH		Plasma base deficit (mEq/l)		Urine pH	
		pre	max	pre	max	pre	max
Term infants							
Group I (N=6)	44–46						
Mean		7 40	7 29	−0.5	−11 5	7 10	5 15
S E.M		0 01	0 02	0 71	0 73	0 06	0.09
Preterm infants							
Group II A (N=6)	37–39						
Mean		7 37	7 24	−1.5	−11 6	7 05	5 45
S E.M		0 01	0 01	0 76	0 41	0 06	0.15
Group II B (N=7)	37–39						
Mean		7 38	7 28	−1.2	−10 2	6.93	5 06
S E.M		0 01	0 01	0.86	0 37	0.03	0 08
Group II C (N=7)	37–39						
Mean		7.37	7 26	−1 4	−12.8	6.74	5.34
S E.M		0 01	0 01	0.84	0.79	0 05	0 17

H^+_{max} (μEq/min/1.73 m²) pre	max	H_{TA} pre	max	H_{NH_4} pre	max	$HCO_{3\,exc}$ pre	max
13.8 0.27	85.8 3.3[illegible]	6.2 0.20	28.6 ** 0.92	7.6 0.15	57.2*** 3.37	–	–
12.3 1.20	44.6 5.00	6.0 0.47	14.6 2.57	6.3 0.94	30.3 2.67	–	–
19.1** 1.54	50.7 6.47	11.6** 1.09	23.4* 4.10	7.6 0.52	28.8 3.65	0.85 0.47	–
25.8 ** 0.91	52.4 6.91	17.7** 1.11	28.1 3.90	8.8 0.30	24.8 3.[illegible]1	1.22 0.30	–

H_{max} (μEq/min/1.73 m²) pre	max	H_{TA} pre	max	H_{NH_4} pre	max	$HCO_{3\,exc}$ pre	max
23.1 1.12	83.0* 5.00	9.1 0.41	42.0*** 2.83	10.0 0.73	41.0* 3.21	–	–
18.8 2.05	71.1 6.01	8.3 0.74	34.0 4.05	10.5 1.09	37.1 2.34	–	–
22.5** 1.88	78.8 4.00	12.6 0.84	35.9 2.68	9.7 0.39	42.9 2.[illegible]	–	–
27.0** 0.98	74.5 6.39	17.4 0.69	39.4 3.38	9.7 1.13	35.0 3.01	–	–

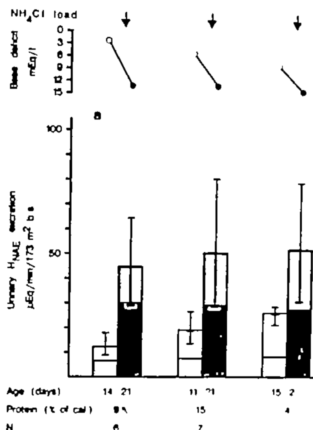


Fig. 2a. Influence of protein intake on plasma deficit and urinary H-excretion before and during induced acidosis in preterm infants at 1–3 weeks of postnatal life, i.e. 34–36 weeks of gestational age. Symbols: Preloading and maximum plasma base deficit (mean) ○—●; urinary H_{NAE} (mean and range) [; H_{TA} (mean) ◻; and H_{NH} (mean) ◼.

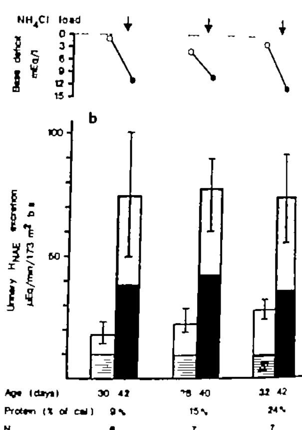


Fig. 2b. Influence of protein intake on plasma base deficit and urinary H-excretion before and during induced acidosis in preterm infants at 4–6 weeks of postnatal life, i.e. 37–39 weeks of gestational age. Symbols as in Fig. 2a.

relation to protein intake (9%, 15% and 24%, respectively, of total calorie supply, see Table 2). The plasma base deficit values as well as the renal H^+-excretion values for the 3 subgroups are shown in Fig. 2a (at 1–3 weeks of postnatal age = 34–36 weeks of gestation) and in Fig. 2b (at 4–6 weeks of postnatal age = 37–39 weeks of gestation).

Preloading values at 1–3 weeks of postnatal age. The H^+-excretion is significantly higher in infants fed formula B (for H^+_{NAE} $p<0.01$, for H^+_{TA} $p<0.001$) and formula C (for H^+_{NAE} $p<0.001$, for H^+_{TA} $p<0.001$ and for $H^+_{NH_4^+}$ $p<0.05$) in comparison to infants fed formula A (Fig. 2a and Table 3a).

At 1–3 weeks of postnatal age some of the preterm babies (3 of 7 fed formula B and 5 of 7 fed formula C) have base deficit values exceeding −8.0 mEq/l indicating a so-called late metabolic acidosis (16). The occurrence of $HCO_3^-{}_{NBE}$ in the preloading urine specimens in these babies (Table 3a) is characteristic of the urinary bicarbonate leak found in babies with this acid base disturbance (27).

Maximum values during induced acidosis at 1–3 weeks of postnatal age. No statistically significant difference in the H^+_{NAE}- and $H^+_{NH_4^+}$-excretion is found between the three subgroups of preterm infants (Fig. 2a and Table 3a). In infants fed formulas B and C the H^+_{TA}-excretion is slightly higher ($p<0.05$).

Preloading values at 4–6 weeks of postnatal age. Also at this age the preloading H^+-excretion values differ between the three subgroups being higher in infants fed formula B (for H^+_{NAE} and H^+_{TA} $p<0.01$) and

formula C (for H^+_{NAE} and H^+_{TA} $p<0.001$) as compared to infants fed formula A (Fig. 2*b* and Table 3*b*). However no difference was found with respect to the $H^+_{NH_4}$-excretion. At this age all infants were in normal acid-base balance.

Maximum values during induced acidosis at 4–6 weeks of postnatal age. No difference is found between the three subgroups of preterm babies with regard to H^+_{NAE}, H^+_{TA} and H_{NH_4}-excretion. Although the maximum H_{TA}-excretion during induced acidosis is also slightly higher in infants fed high protein formulas the increase from preloading to maximum H^+_{TA} values is the same in the three dietary groups.

Summarizing, with increasing age there is a rapid rise of the renal H-excretion prior to and during an acid load irrespectively of previous amounts of dietary protein intake. The *maximum* renal H^+-excretion values during induced acidosis at 4–6 weeks of postnatal age are equal in all three dietary groups of preterm infants as shown in Fig. 2*b* and Table 3*b*.

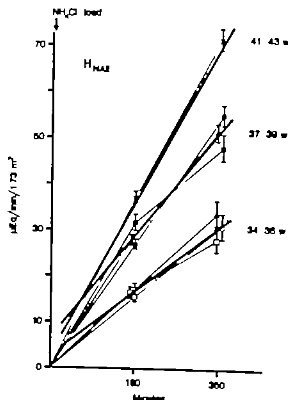


Fig. 3. Increment of excretion of urinary H_{NAE} over preloading control levels during induced acidosis. Mean and S.E.M. of actual incremental values connected by thin lines. Solid lines calculated by least squares with equations for linear regressions.

Weeks of gestation.

34–36	$H_{NAE} = 2.6201 + 4.6883\,X$ $= 0.5532$.
37–39	$H^+_{NAE} = 6.1003 + 7.6215\,X$ $r = 0.7177$
41–43	$H_{NAE} = 2.6167 + 11.5611\,X$ $r = 0.9316$.

(X = time in minutes after acid load. r = coefficient of correlation.) Symbols: Term infants at 41–43 weeks of gestation. Group I X. Preterm infants at 34–36 and 37–39 weeks of gestation. Group II A △ ▲, Group II B ○ ●, Group II C □ ■.

B. *Cumulative Excretion Rate of H after Induced Acidosis*

1. *Re gestational and postnatal age*

Fig. 3 shows the cumulative excretion of urinary H_{NAE} in preterm infants studied at 34–36 and again at 37–39 weeks of gestation and also the term infants studied at 41–43 weeks of gestational age. The values constitute the increment over the preloading control values at 180 and 360 minutes respectively. The means within each group and subgroup of infants are connected by thin lines. The linear regressions (indicated by solid lines) of each gestational age period have been calculated by least squares.

The low cumulative excretion rate in preterm infants is demonstrated by the less steep slope of the solid lines for these infants. The value for the slope of the regression lines is 4.6883 at 34–36 weeks, 7.6216 at 37–39 weeks and 11.5611 at 41–43 weeks of gestational age (for calculation see text of Fig. 3).

Thus with increasing age there is not only an increase of the maximum level of H_{NAE}-excretion after induced acidosis, but also an enhanced *cumulative* H^+-excretion rate.

2. *Re dietary protein intake*

In infants with a low protein intake (groups I and II A) and those with a moderate pro-

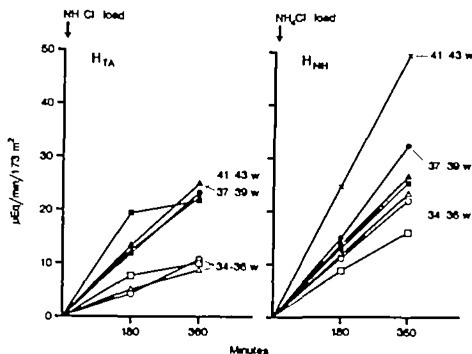


Fig 4 Increment of excretion of urinary H_{TA} and H_{NH_4} over preloading control levels during induced acidosis. Mean of actual incremental values within each group connected by thin lines. Symbols as in Fig 3

tein intake (group II B) the cumulative renal H^+-excretion rates are actually linear as shown in Fig 4 In contrast to this the mean values of the infants with a higher protein intake (group II C) differs from those of the 2 other dietary subgroups there is an enhanced cumulative excretion of H^+_{TA} during the first 180 minutes after the acid load at 360 minutes no difference is found However this difference at 180 minutes is statistically significant only in the babies of 37–39 weeks of gestational age (for H^+_{TA} $p < 0.05$ when comparing group II C with II A) It should be noted that the lowest mean plasma base deficit level during induced acidosis is of the same order in all three groups at 37–39 weeks of gestation as shown in Fig. 2*b* This indicates that the increased H^+_{TA}-excretion in group II C is not a consequence of a low blood pH and plasma base deficit level per se

It should be observed that the cumulative excretion rates of $H^+_{NH_4^+}$ are linear and increasing uniformly with gestational age in all preterm infants irrespectively of the dietary protein intake

DISCUSSION

General aspects

In fetuses and young infants there is a nephronic heterogeneity with glomerular preponderance (7) It has been shown by Fetterman et al (8) that during maturation of the kidney there is a rapid tubular growth accounting for most of the increase of kidney size in fetal life Concomitantly a process of enzymatic maturation is taking place (29)

The renal hydrogen ion excretion capacity in early infancy as judged by acid loading tests has previously been studied in a limited number of infants (5 9 11 14 15 20 23 25) The wide range of urinary excretion values obtained in these investigations are mainly due to varying gestational and postnatal ages of the infants and to a minor extent also to slight differences in the procedures and techniques used In none of them have the studies been repeated in the same babies at different intervals as performed in the present investigation

Influence of maturity and age

It is reasonable that the low H^+_{NAE}-excretion capacity observed in preterm infants during the first weeks of postnatal life—in comparison to term infants of the same postnatal age—is related to their short gestation. Nevertheless a rapid increase of the renal tubular acidification in the postnatal period is found in these babies. When approaching full gestation this capacity is only slightly lower in preterm babies than in term babies 1–3 weeks of age.

The renal hydrogen ion excretion capacity—as measured by induced acidosis with the short term single dose NH_4Cl loading test at one to two weeks of postnatal age—has been studied by Hatemi and McCance in 8 term infants (14), by Kerpel-Fronius et al. in 7 premature infants (15) and by Sulyok et al. in 10 term and 33 premature infants (25). The mean gestational age of the premature infants in the lastmentioned study was 34 gestational weeks, thus comparable to the gestational age of the preterm infants of the present investigation. The maximum renal H^+_{NAE}-excretion observed in the abovementioned study (for term infants 52 μEq/min/1.73 m² and for premature infants 28 μEq/min/1.73 m²) is somewhat lower than that observed at 1–3 weeks of postnatal age in the present investigation (for term infants 85.5 μEq/min/1.73 m² and for preterm infants 44.6 μEq/min/1.73 m²).

No reports have been published with the short term acid loading test at 4–6 weeks of postnatal age. Infants of this age have however been studied by longterm $CaCl_2$-loading tests (for 3 to 21 days) in a dosage of 10.7 mEq/kg/day (23). This study comprised 4 smaller and 3 larger premature infants aged 26 to 49 days. The maximum H^+_{NAE}-excretion obtained—40.4 μEq/min/1.73 m² in the smaller and 85.8 μEq/min/1.73 m² in the larger prematures—is of the same order as that in the present investigation with maximum H^+_{NAE}-excretions ranging between 50.2 and 90.2 μEq/min/1.73 m² in the preterm infants. This indicates that the short term acid loading test permits an accurate measurement of the renal maximum H^+-excretion capacity, thus not necessitating a longterm acid loading of the baby.

Infants more than 4–6 weeks of age have been studied with the short term NH_4Cl loading test by Edelman et al. (5). The maximum renal H^+_{NAE}-excretion values found by these authors in 6 infants aged 1 to 12 months ranged from 85 to 190 (mean 119) μEq/min/1.73 m². Thus in comparison to the findings of the present investigation in term infants at 4–6 postnatal weeks (44–46 gestational weeks)—maximum H^+_{NAE} ranging from 70.4 to 101.4 (mean 83.0) μEq/min/1.73 m²—older infants have only a moderately higher renal hydrogen ion excretion capacity.

The results of the present investigation indicate that there is a rapid maturation of the renal acidification capacity already within the first postnatal weeks. Furthermore it is apparent that the enhancement in maximum H^+_{NAE}-excretion after induced acidosis is initially more closely related to gestational than to postnatal age (Fig. 1). This is most conspicuous for $H^+_{NH_4^+}$, showing that the (linear) cumulative excretion rate increases with increasing gestational age (Fig. 4). This increase is probably related to maturation: it is known that the gluconeogenic capacity of the kidney increases with growth (29) and that increased renal gluconeogenesis is associated with increased ammoniagenesis (1, 21).

Influence of dietary protein content

Controversial results regarding the influence of dietary protein intake upon postnatal development of renal functions have been reported. Thus in a study of 8 premature infants fed high protein (5.0 g%) and low protein (2.5 g%) diets for about four weeks

Edelman and Wolfish (6) observed in the high protein group at the end of this period an increased titratable acid and ammonium excretion before and after NH_4Cl load The gestational age and maturity of their premature infants is not stated in their report Similar observations have been made in older infants (2 to 6 months of age) by Fomon et al (9)

Contrary to this Moore et al (19) measuring the renal response to NH_4Cl loads in normal and malnourished suckling rats found that—although the larger infants had statistically higher glomerular filtration rate and kidney weights—there was no difference in the excretion of titratable acid and ammonium These studies indicate that the maturation of specific tubular functions in suckling rats is related rather to age and may proceed independent of kidney size dietary intake and excretory needs

In the present investigation the preloading H^+-excretion values both at 1–3 and 4–6 weeks of postnatal age were generally higher in preterm infants fed the high protein formulas this refers to the H^+_{TA} component (Figs 2*a* and 2*b*) During induced acidosis at 1–3 weeks of age the maximum H^+_{TA} excretion was also slightly higher in these infants ($p<0.05$ Table 3*a*) The rise of H^+_{TA} from preloading to maximum values at 360 minutes was not significantly higher in these infants than in infants fed the low protein formula (Fig. 4) However the corresponding rise at 180 minutes in preterm infants 4–6 weeks of age (=37–39 gestational weeks) was significantly higher in those fed the highest protein formula than in the two other subgroups (Fig. 4) These findings indicate that after a few weeks on a high protein intake there is an enhancement of the rate of the H^+_{TA}-excretion This is possibly a consequence of the high dietary osphate content which increases the exction of urinary titratable acid (14 18) capacity to utilize the increased phosphate ntake for titratable acid formation may indicate that an increased ability to secrete H^+ in the urine has developed in the infants fed the highest protein formula In the study by Edelman and Wolfish (6) not only the H^+_{TA} but also the $H^+_{NH_4^+}$-excretion values were increased in the high protein group probably because their infants got this diet for a longer period of time than the infants of the present investigation

Thus the magnitude of work load imposed upon the kidney by the diet seems—after a certain adaptation period—to influence the rate of maturation as observed both in the clinical investigations and also in some animal experiments (12 24) However this does not imply that high protein intake is optimal for preterm infants during the first weeks of life because several of the infants on the high protein diet (subgroup II C) developed disturbances of the blood acid-base homeostasis so-called late metabolic acidosis (16) Many preterm infants with this acid-base disorder recover spontaneously (26) primarily due to the rapidly increasing hydrogen ion excretion capacity This development may apparently to a certain degree respond to increased excretory needs

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PNEUMOENCEPHALOGRAPHY IN NON PROGRESSIVE ATAXIC SYNDROMES

A Study of 26 Children and Adolescents

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ABSTRACT Bergström K and Sanner G (Departments of Diagnostic Radiology and Paediatrics University Hospital Uppsala, Sweden) Pneumoencephalography in non progressive ataxic syndromes. A study of 26 children and adolescents. Acta Paediatr Scand 63:732, 1974.—Twenty-six patients with a congenital non-progressive ataxic syndrome underwent pneumoencephalography (PEG) The patients were classified clinically into four groups: (I) The dysequilibrium syndrome (DES) (II) The dysequilibrium syndrome with cataract (III) Transitional form (TF) (IV) Simple ataxia (SA). In patients with signs of dysequilibrium different PEG changes were found 16 of the 21 patients had cerebellar abnormalities. In all but one of the evaluable cases with signs of DES and cerebellar abnormalities on PEG a small antero-superior part of the vermis was characteristic which might be of patho-genetic importance None of the 5 patients with SA had a small cerebellar vermis. It was suggested that PEG may be of diagnostic help in infancy in presumptive ataxic conditions, but is neither of prognostic importance nor of diagnostic help for delineating specific hereditary forms.

KEY WORDS: Congenital ataxia, dysequilibrium syndrome Marinesco-Sjögren syndrome pneumoencephalography cerebellar abnormalities

Within the concept of cerebral palsy different clinical syndromes have been delineated the common denominator being a persisting qualitative motor disorder appearing before the age of three years due to a non-progressive interference with development of the brain (15) The aetiology and pathology differ even within the same clinical syndrome of cerebral palsy (6 11) Consequently the pneumoencephalographic findings in patients with cerebral palsy are variable and abnormalities of the same central cerebral structures are common irrespective of the clinical type of the cerebral palsy syndrome (37)

During the last decades a more accurate clinical delineation of different syndromes of ataxic cerebral palsy has improved the possibilities of evaluating these cases also from aetiological and patho-genetic aspects (9 10 11 35) Among the syndromes that of simple ataxia (SA) and the dysequilibrium syndrome (DES) are of particular interest for studying the occurrence of various prenatal factors which dominate within these groups (9 10 11 35)

PEG studies constitute one way of obtaining more information on the brain morphology even if the exact nature of the observed pathological findings often remains obscure However the radiological literature concerning patients with ataxic syndromes is scanty and is mostly concerned with progressive ataxic disorders in adults (4 17 39)

PEG findings in nine patients with DES have previously been reported by Hagberg et al (9) Although various abnormalities were

Table 1 *Clinical data for the present series*

Group	Case No	Sex	Age at PEG (y)	Cranial nerve signs
I	1	M	11	Convergent squint + VIth nerve palsy
(DES)	2	M	2 3/4	Convergent squint
	3	M	5	
	4	M	10	
	5	M	8	Congenital ptosis
	6	F	2	Convergent squint + VIth nerve palsy
	7	M	11	Convergent squint
	8	M	15	Convergent squint
	{9	F	4	
	{10	M	1/4	
	{11	F	2 1/4	Convergent squint
	{12	F	2 1/4	Convergent squint
	13	M	2 1/2	Convergent squint
)ES+	14	M	1 1/2	Convergent squint
ataract)	15	M	25	VIth nerve palsy
	16	F	3	Convergent squint
I	{17	F	2 1/2	Convergent squint
TF)	{18	M	4 1/2	Convergent squint
	19	M	4 1/4	Convergent squint
	20	M	5 1/2	
	21	F	5 1/2	Convergent squint Hypoplasia of nerve II
V	22	F	1/4	
SA)	23	M	6	
	24	F	3/4	Convergent squint
	25	F	1/4	Palsy of nerves V VII, VIII
	26	F	2	

DES = Dysequilibrium syndrome, TF = Transitional form, SA = Simple ataxia, { = siblings.

scored a small cerebellar vermis was found in most cases The present study is an enlargement of the previous series with the aim of establishing whether DES patients have a characteristic pneumoencephalogram suggesting a specific morphological pattern in the brain.

SYSTEM OF NEUROLOGICAL CLASSIFICATION

Earlier classifications of the non-progressive ataxic syndromes according to different neurological signs have been confusing or lacking. In most series of patients with cerebral palsy these patients have been classified either as cases of "ataxia" or in a group of "mixed syndromes" A useful classification was created by Ingram (10, 11), who sub-divided patients with ataxic cerebral palsy into "ataxia" and "*ataxic diplegia*" (ataxia with added diplegic spasticity). However a few patients have as their dominating motor defect a disturbance of posture and equilibrium, i.e. dysequilibrium (3, 15) whereas the signs of dyssynergia (manifested e.g. as intention tremor and dysmetria) may be minimal. A recent study of this disability "*the dysequilibrium syndrome*" (DES), was presented by Hagberg et al. (9). Even though *transitional forms* (TF) between DES and simple ataxia (as defined by Ingram) exist DES in its pure form is a characteristic clinical entity A few patients with DES have spastic diplegia in addition. Hence, there are possibilities of delineating four main syndromes within the concept of ataxic cerebral palsy viz. 1) simple ataxia, 2) ataxic diplegia, 3) the dysequilibrium syndrome and 4) the dysequilibrium syndrome with spastic diplegia.

Some of the patients with DES also exhibit cataract and mental retardation. This triad of signs (ataxia, cataract, mental retardation) allow them to be classified as cases of "the Marinesco-Sjögren syndrome" (36). Except for the cataract these patients do not differ clinically from other patients with DES. For reasons discussed elsewhere (9 34) the Marinesco-Sjögren syndrome as a clinical entity can be questioned. In the present study the patients with cataract are discussed separately in order to compare them with other patients reported in the literature.

CLINICAL MATERIAL

The present series consists of 26 patients with a congenital non-progressive ataxic syndrome (Table 1). Their ages at the time of the PEG varied from 3 months to 25 years (Table 1).

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During the last decades a more accurate clinical delineation of different syndromes of ataxic cerebral palsy has improved the possibilities of evaluating these cases also from aetiological and patho genetic aspects (9 10 11 35) Among the syndromes that of simple ataxia (SA) and the dysequilibrium syndrome (DES) are of particular interest for studying the occurrence of various prenatal factors which dominate within these groups (9 10 11 35)

PEG studies constitute one way of obtaining more information on the brain morphology even if the exact nature of the observed pathological findings often remains obscure However the radiological literature concerning patients with ataxic syndromes is scanty and is mostly concerned with progressive ataxic disorders in adults (4 17 39)

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Table 2. *PEG findings in the present series*

− = slight reduction, − − = moderate reduction, − − − = pronounced reduction, + = slight enlargement, + + = moderate enlargement, + + + = pronounced enlargement, × = not visualized, 0 = normal

		Infratentorial region						Supratentorial region		
		Cisterna magna			Size of vermis			Lateral ventricles (Evans ratio)		
Group	Case No	Frontal width	Sagittal depth (mm)	Fourth ventricle (height mm)	Ant sup part	Post. sup part	Inf part	Right	Left	Third ventricle (width, mm)
I (DES)	1	+++	+++ (22)	++ (17)	− − −	− − −	− − −	+ (0.38)	+ (0.37)	0 (7)
	2	+++	+++ (15)	+++ (16)	−	×	− − −	0 (0.29)	+ (0.33)	0 (4)
	3	++	++ (16)	++ (15)	− −	×	− −	0 (0..5)	0 (0.25)	0 (4)
	4	0	0 (5)	++ (16)	− − −	×	0	0 (0.29)	0 (0.23)	0 (6)
	5	0	0 (6)	0 (12)	0	0	0	0 (0.26)	0 (0.26)	0 (3)
	6	0	0 (4)	0 (14)	0	×	0	0 (0.23)	0 (0.23)	0 (5)
	7	+++	+++ (23)	+++ (24)	− − −	×	− − −	+ (0.33)	0 (0.30)	0 (7)
	8	0	0 (4)	+ (19)	− − −	0	0	+ (0.42)	+ (0 40)	+ (10)
	9	0	0 (5)	0 (14)	− −	×	0	0 (0.25)	0 (0.24)	0 (4)
	10	0	0 (3)	0 (7)	0	0	0	0 (0.25)	0 (0.25)	0 (6)
	11	0	0 (20)	0 (17)	0	×	0	+ (0.33)	0 (0..5)	+ (10)
	12	0	0 (23)	0 (15)	0	×	0	+ (0.31)	+ (0.32)	++ (12)
II (DES + cataract)	13	+++	+++ (30)	+++ (19)	−	×	− − −	+ (0.37)	+ (0.37)	0 (7)
	14	+++	+++ (22)	0 (12)	×	×	− −	0 (0.25)	0 (0.25)	0 (5)
	15	+++	+++ (19)	+++ (26)	− − −	− − −	− − −	0 (0.30)	0 (0.30)	0 (7)
	16	Large		0	×	×	?	0	0	0
III (TF)	17	+++	+++ (23)	+++ (19)	− − −	− − −	− − −	0 (0 29)	0 (0.29)	0 (7)
	18	+++	+++ (30)	+ (15)	−	×	− − −	+ (0.33)	+ (0 33)	0 (7)
	19	0	0 (7)	0 (10)	0	×	0	0 (0 30)	0 (0.30)	+ (10)
	20	0	++ (16)	+ (17)	− −	− −	−	0 (0.29)	0 (0.29)	0 (6)
	21	+++	+++ (21)	+ (14)	− − −	− − −	− − −	0 (0.28)	0 (0.28)	0 (5)
IV (SA)	22	0	0 (3)	0 (8)	0	0	0	+ (0.33)	+ (0.33)	0 (5)
	23	0	0 (5)	0 (14)	0	×	0	+ (0.39)	+ (0.39)	++ (12)
	4	0	0 (9)	0 (12)	0	×	0	+ (0.39)	+ (0.41)	+ (8)
	25	+++	0 (10)	0 (11)	0	0	0	+ (0.32)	+ (0.32)	+ (9)
	26	0	0 (10)	0 (9)	0	0	0	0 (0.28)	0 (0.28)	0 (7)

concavity of the anterior medullary velum or of the nodulus.

The cisterna magna varies normally in size and configuration, the height in an adult material ranging from 15 to 55 mm (20). A high cisterna magna alone is therefore considered insignificant. However enlargement of Magendie's foramen and widening of the vallecula strengthen the pathological significance of a large cisterna magna (31). Furthermore sagittal enlargement of the central parts and enlargement of the width seen in the antero-posterior projection are significant, indicating smallness of the cerebellum. We therefore evaluated the configuration of the cistern, and assessed the sagittal dimension and the frontal width. Eisenman et al (8) found the normal sagittal dimension to be 9.25±3 76. No normal values of the frontal width seem to have been reported. In children the size of the cisterna magna increases somewhat during the first two years of life and thereafter remains stationary (5 8).

The supracerebellar cistern is seen as a narrow layer above the cerebellar vermis (20). Its depth may vary but wide sulci are an abnormal finding (31). In the majority of normal individuals gas does not enter between the folia of the cerebellar vermis (4).

The pontocerebellar cisterns. Their appearance in children was investigated by Carlsson & Lodin (5). The width of the cisterns was found to decrease somewhat after the age of two years. For children below four years of age the mean value was 7 0 mm and for those above four years of age 5 2 mm.

The pontine cistern. As a small pons often accompanies a small cerebellum (38), we also made an assessment of the pontine cistern. This cistern remains at about the same size during the growth period (5 8). Eisenman et al. (8) reported a variation of the sagittal depth from 3 to 12 mm, with a mean of 5.6± 1 76 mm. *Size of the cerebellum.* From the size and configuration of the cisterns and fourth ventricle information on the state of the cerebellum is obtained. The vermis is best evaluated in sagittal tomograms. The superior parts of the vermis may be seen outlined by gas in the supracerebellar cistern. The infero-posterior part of the vermis may be evaluated, judging the central parts of the cisterna magna and the width of the vallec



Fig. 1. Case 5. Sagittal midline tomogram (as in Figs. 3–7). Normal anatomy. Schematic drawing with vermis shadowed (as in Figs. 3–7). *Antero-superior part of vermis*: 1. Lingula. 2. Lobulus centralis. 3. Culmen. *Postero-superior part of vermis*: 4. Declive. 5. Tuber. *Inferior part of vermis*: 6. Pyramis. 7. Uvula. 8. Nodulus. *a* Cisterna magna. *b* Supracerebellar cistern. *c* Pontine cistern. *d* Fourth ventricle. *e* Primary fissure. *f* Anterior medullary velum. *g* Vallecula (and cerebellar tonsil). *h* Magendie's foramen.

All patients were clinically examined and neurologically classified by Dr Bengt Hagberg[1] and/or one of the authors (G. S.). The distribution according to clinical classification (as defined above) is illustrated in Table 1. Sixteen patients had DES (without spasticity), four of them had associated cataract, five had SA and five were considered to have a transitional form. Three patients were intellectually normal and 23 had mental retardation.

Cranial nerve abnormalities, especially convergent squint, were noted in 18 of the 26 patients (Table 1). Four patients with DES had a probably postnatal cataract.

There were three pairs of identically disabled siblings of whom one pair (cases 11 and 12) were monozygotic twins. In only one patient (case 8) were evidence of perinatal asphyxia recorded. In the other 25 patients the aetiology of the ataxia was unknown, but in the DES group it was assumed from previous studies (34) to be genetically determined with an autosomal recessive inheritance.

METHODS

PEG procedure

All patients were examined under general anesthesia after lumbar puncture, mainly according to the technique described by Lindgren (21). In 21 patients sagittal tomography of the posterior fossa was performed, and in some of them also frontal tomography. Autotomography was performed in three patients. In the remaining two the examination did not include any tomographic study.

No complications occurred, but in a few patients the ataxia was temporarily aggravated for 2–3 days after the PEG.

Evaluation of the PEG findings

The normal variation of the different parts of the encephalogram is large, sometimes making the judgement of abnormality hazardous. This is especially true for the cisterns of the posterior fossa. In children the development of the cisterns of the posterior fossa has been studied by Carlsson & Lodin (5) and Eisenman et al (8). The normal variations of some of the cisterns are very wide. In the individual patient, therefore, an evaluation must include both the dimensions and configuration of the structures studied.

All pneumoencephalograms were assessed by one of the authors (K. B.). In case 16 the final appraisement was incomplete as the films were unfortunately lost after a preliminary evaluation.

The variations in size and configuration that are considered normal for the posterior fossal cisterns and the ventricular system are described below.

The infratentorial region

The fourth ventricle reaches adult size by the age of two years (22). The normal range is large, the sagittal height varying from 11 to 21 mm. However, in some cases we regarded a height below 21 mm as pathological because of an abnormal configuration, especially

[1] Previous Assoc. Prof. in Paediatric Neurology in Uppsala, since 1971 Prof. of Paediatrics in Gothenburg.





Fig. 4. Case 15. The whole vermis is extremely small.

regarding signs of atrophy and other abnormalities. The widths of the sulci are large prior to 1 months of age but after this age are the same in adults (32).

RESULTS

The results of the PEG examinations are presented in Table 2. The patients are tabulated in four groups according to their clinical classification.

In *group I* (pure DES) comprising 12 patients the findings varied. Three patients (cases 5, 6 and 10) had a completely normal PEG (Fig. 1). In seven patients (cases 1, 2, 3, 4, 7, 8 and 9) the main pathological findings were in the cerebellar area. In four of them (cases 1, 2, 3 and 7) a marked reduction in size of all cerebellar parts was noted (Fig. 2). The superior part of the vermis was not vis-





Fig. 5. Case 17. All parts of the vermis are very small, with irregular and nodular lobuli.





Fig. 2. Case 1. All parts of the vermis are very small.

ula and Magendie's foramen. In our study we tried to determine whether the antero-superior, the postero-superior and the inferior parts of the vermis were diminished or not. The size of the cerebellar hemispheres is more difficult to evaluate, but a small size is indirectly indicated by an increased width of the lateral parts of the cisterna magna and fourth ventricle and also by enlarged pontocerebellar cisterns.

The supratentorial region

The sizes of *the lateral ventricles* were judged by means of Evans' index, i.e. the ratio between the width of *the frontal horns* and the inner cranial diameter (mean value in adults 0.23, ranging from 0.16 to 0.29). This index is also useful in children (13). We regarded an index over 0.30 as pathological. However, slightly higher values, mean 0.30, range 0.24–0.33, have been reported for normal children during the first two years of life (32). A difference between the two sides was considered abnormal.

The third ventricle remains approximately the same size from birth (22, 3). We regarded a width of 8 mm or greater as pathological (26).

The cortex of the cerebral hemispheres was evaluated





Fig. 3. Case 8. Selective diminution of part of the antero-superior portion of the vermis (lingula and lobulus centralis).

the finding of an enlarged cisterna magna and/or fourth ventricle (19). In his other patients with ataxia associated with spasticity or athetosis the PEG findings were different

In the *present study* the patients were classified into four groups

In *group I* (DES without cataract) the PEG findings were not uniform. However in all the seven patients with cerebellar PEG abnormalities a small antero-superior vermis was found This may offer a satisfactory pathogenetic explanation for the pronounced postural dysfunction in these cases Three patients had a completely normal PEG In view of the severe disablement in these patients this finding seems remarkable However maldevelopment on the cellular level, as found in a DES case by Berg et al. (2) could be a reasonable explanation.

In *group II* (DES with cataract, classifiable as cases of the Marinesco-Sjögren syndrome) the findings indicate small cerebellar hemispheres and a small cerebellar vermis (Fig. 4). In previously reported cases of the Marinesco-Sjögren syndrome subjected to a PEG various abnormalities have been found (1 27) Two similar autopsy cases have been reported (23 40) with abnormalities consisting of cortical atrophy of the neocerebellum, parts of the vermis and pons The PEG findings in our four cases of DES with cataract thus fit with the above-mentioned autopsy findings but not with earlier PEG reports The PEG appearance of the cataract cases cannot be differentiated from the findings in four of our pure DES cases in group I

In *group III* (TF cases) the findings were of the same varying type as in the pure DES group It is not surprising that these patients with signs of both DES and SA have PEG changes some of which are very similar to those found in the pure DES group

Group IV (SA cases) represents basically the same neurological group as "the bilateral neocerebellar syndrome presented by Leany (18 19) In only one patient in this group was a cerebellar abnormality found and this consisted of small cerebellar hemispheres I e the same abnormality as Leany (18) observed in all cases in his series In our series four patients of this group had no macro-anatomic cerebellar abnormalities at all Although our group of cases is small it can be concluded that small cerebellar hemispheres are not found in every case of simple ataxia

Four of our patients in group IV had dilatation of the lateral ventricles In two of these the third ventricle was also enlarged The results of our study are in agreement with the opinion of Ingram (12) that in ataxic cerebral palsy various forms of PEG features can be seen.

In the *whole material* of 26 patients 17 had cerebellar PEG abnormalities In the remaining nine patients either a completely normal PEG or abnormalities only on the supratentorial level were found. A connection between the ataxic signs observed and the cerebellar PEG abnormalities found cannot be presumed *a priori* Firstly in adults with no signs of ataxia during their lives severe cerebellar abnormalities even total aplasia, have been found at autopsy (29 33) Secondly not all patients in our series had cerebellar PEG abnormalities However the high frequency of cerebellar abnormalities in this series makes such a connection probable

Whether cerebellar abnormalities found on PEG represent *maldevelopment or atrophy* has been discussed The abnormalities of the fourth ventricle and cisterna magna are unspecific and obviously of the same appearance in the two conditions (24) Some authors have regarded widening of cerebellar sulci mainly as a sign of atrophy while an irregular and nodular convexity as found in our case 17 (Fig. 5) has been postulated to characterize maldevelopment (24 38) However few comparative studies between PEG and autopsy on hypoplastic cerebellums have been reported and other authors consider PEG differentiation between atrophy and hypoplasia to be impossible (7)

ualized in case 2 but concavity of the anterior medullary velum indicated smallness of the antero-superior part of the vermis In three patients (cases 4 8 and 9) an obvious reduction of the antero-superior part of the vermis was the only cerebellar abnormality found (Fig. 3) The lateral ventricles were asymmetrical in case 4 and dilated in cases 1 2 7 8 11 and 12 three of whom also had a dilated third ventricle (cases 8 11 and 12)

In *group II* (DES with cataract) comprising four patients the findings were similar In all patients the cerebellar hemispheres were small The inferior part of the vermis was diminished in three patients (cases 13 14 and 15) (Fig 4) and was not evaluated in case 16 The antero-superior part of the vermis was reduced in size in cases 13 (judged from the configuration of the anterior medullary velum) and 15 but could not be evaluated in cases 14 and 16 The postero-superior part of the vermis was small in case 15 and unevaluable in cases (13 14 and 16

In *group III* (TF) comprising five patients one patient (case 19) had enlarged ponto-cerebellar cisterns as the only sign of cerebellar abnormality A slightly enlarged third ventricle was also found In two siblings (cases 17 and 18) the cerebellar hemispheres and the inferior part of the vermis were diminished (Fig 5) The rest of the vermis was also very small in case 17 and reduced in case 18 (judged from the configuration of the anterior medullary velum) In case 18 the lateral ventricles were slightly dilated In case 20 the cerebellar hemispheres seemed to be of normal size whereas the vermis was diminished especially its antero-superior part In case 21 all parts of the cerebellum were small

In *group IV* (SA) comprising five patients the cerebellum was abnormal in only one case (case 25) This patient had small cerebellar hemispheres but a normal-sized vermis In one patient (case 26) the PEG was normal In four patients (cases 22 23 24 and 25) the lateral ventricles were slightly enlarged In three of them (cases 23 24 and the third ventricle was also enlarged

The following additional abnormalities w found sporadically in patients from differe groups of this series

In five patients (cases 2 13 14 18 a 25) the pontine cistern was enlarged In of them there were also signs of small cer bellar hemispheres In four patients (case 13 15 18 and 19) the pontocerebellar ci terns were enlarged three of them als showed an increase in width of the cisterna magna in the anteroposterior view

DISCUSSION

The anatomy of different macroscopic structures of the cerebellum can be well visualized pneumoencephalographically with the use of a specially modified technique (21) The great value of tomography has been stressed by several authors (4 17 39) The vermis, in particular may be delineated in detail in a sagittal midline tomogram (39) even though the postero-superior part often is not outlined by gas posterior to the primary fissure. Our study confirms that with a careful technique including tomography the macro-anatomy of most parts of the cerebellum can be evaluated

Earlier series of ataxic patients examined with PEG have mainly consisted of adults suffering from progressive ataxic disorders particularly alcoholic encephalopathy (4 17 39) Different types of PEG abnormalities mainly a large cisterna magna and/or fourth ventricle have previously been reported in children with congenital ataxic syndromes (14 16 30) However in these reports the syndrome has not always been defined neurologically and in most reports the description of the PEG findings is incomplete

Lesny (18) reported a PEG study of a series of children with clinically classified ataxic disorders In 19 patients with a bilateral neocerebellar syndrome he found symmetrical diminution of the cerebellar hemispheres This statement was based on

syndromes is not unequivocal During the first year of life PEG abnormalities can give valuable information for differential diagnosis in patients with the 'floppy infant syndrome However from prognostic and therapeutic points of view the PEG appearance of the cerebellum is unimportant To judge from our clinical experience patients with a normal PEG and others with a markedly diminished cerebellum can have an identical developmental profile

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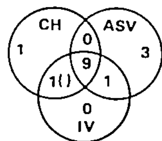


Fig 6 Interrelations of cerebellar PEG abnormalities in the patients with signs of DES (groups I II and III) IV=Inferior part of vermis small ASV=Antero-superior part of vermis small CH=Cerebellar hemispheres small. (x)=ASV not possible to evaluate

From a clinical point of view the non-progressive course in our patients is in favour of maldevelopment but it is also compatible with cerebellar atrophy occurring prenatally or during early life In siblings who during life had had a congenital non progressive ataxia Norman (28) found congenital atrophy of the granular layer at autopsy Cerebellar atrophy may also be caused by different exogenous factors known to affect the cerebellum in the prenatal and neonatal period (25) However in our series no such influence by exogenous factors during the pre or neo-natal period was suggested by the patients histories

One pair of similarly disabled siblings in our series showed almost identical cerebellar abnormalities on PEG The parents were second cousins This makes a prenatal or neonatal exogenous atrophic brain damage process less likely than a recessively inherited maldevelopment in these patients Furthermore hypoplasia of different parts of the vermis some times inherited in an autosomal recessive mode has been reported in the neuropathological literature (14)

Thus no definite conclusions can be drawn from the PEG studies as to whether the observed cerebellar PEG abnormalities represent maldevelopment or atrophy According to reported autopsy findings in similar patients atrophy seems probable in our group II In the rest of the series the clinical and genetic data make maldevelopment or foetal atrophy (28) plausible

The difficulties of *correlating PEG findings in ataxic patients to specific clinical syndromes* have been testified by several authors (12) We have encountered the same difficulties However in 13 of the 16 patients with DES in some form (group I II and III Fig 6) and cerebellar abnormalities on PEG the antero-superior part of the vermis was small, in two of the 16 it could not be evaluated Lesny (18) on the other hand in his 19 patients with a symmetrical neocerebellar syndrome (corresponding to our group IV SA) found only one patient with an enlarged supracerebellar cistern he regards this patient as having a transitional state between DES and SA (19) Neither was any patient with a small vermis found in our group IV with SA

To conclude the findings in the present study support the view of Hagberg et al (9) that dysfunction of the cerebellar vermis is of special pathogenetic importance for the development of the clinical sign dysequilibrium To explain all the components of the dysequilibrium syndrome developmental defects of lesions on different functional levels of the brain must be considered

In a recent study the *heredity* of the dysequilibrium syndrome (with or without cataract) was found likely to be autosomal recessive (34) In that report the possibility that the syndrome has clinico-genetic heterogeneity is discussed The varying PEG feature found in the present investigation may suggest that DES is not a neuropathological and thus not a genetic entity However different expressions of cerebellar maldevelopment or atrophy as seen in our series might well be caused by one single gene abnormality (14) Nevertheless the possibility of being able to use PEG for differentiating the hereditary cases of congenital ataxic syndromes from cases with other pre or perinatal causes seems small

The *indication for PEG* in congenital ataxic

Acta Pædiatr Scand 63 743–749 1974

NEONATAL HYPOGLYCAEMIA AND MATERNAL TOXAEMIA

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ABSTRACT Koivisto, M., Jouppila, P (Departments of Paediatrics, and Obstetrics and Gynaecology University of Oulu Oulu, Finland). Neonatal hypoglycaemia and maternal toxaemia. Acta Paediatr Scand, 63:743 1974.—The incidence of hypoglycaemia was determined in 131 newborn infants of toxaemic mothers. The blood glucose level was determined three times daily during the first 3 days of life.

Blood glucose levels <20 mg/100 ml occurred in 24% and levels <30 mg/100 in 65% of the whole series. The incidence of hypoglycaemia was highest, 61% <20 mg/100 ml, among the infants born to toxaemic mothers with low urinary oestriol; the corresponding incidence for mothers with normal oestriol was 19% The incidence was 35% when the maternal toxaemia was severe, and 16% when it was mild. SFD infants had an incidence of 37% compared with 19% for infants of normal birthweight. The relative weight of the placenta did not correlate with the occurrence of hypoglycaemia. Symptomatic hypoglycaemia was noted in 19 infants.

The reduced energy reserves in newborn infants of toxaemic mothers with severe pre-eclampsia and/or low urinary oestriol level and in infants with intrauterine growth retardation, together with increased utilization of carbohydrates result in a high incidence of neonatal hypoglycaemia. An added stress, such as birth hypoxia and late initiation of feeding, increase the likelihood of hypoglycaemia.

KEY WORDS: Hypoglycaemia, maternal toxaemia, oestriol

Neonatal hypoglycaemia occurs most frequently in infants having a low birthweight and in infants born to toxaemic and diabetic mothers (4 12 13 20 23) It is often associated with other neonatal disorders such as asphyxia, congenital infections haemolytic disease or primary cerebral damage (2 4 7 21) It has recently been confirmed that hypoglycaemia also occurs in normal newborn infants not included in any risk group (13 16)

The incidence of neonatal hypoglycaemia in general newborn nurseries is difficult to estimate because of the different criteria used to define hypoglycaemia, the dissimilar populations and the different feeding systems in the various nurseries Reported, incidence from different types of newborn nurseries has varied from 1 to 5% (3 9 17). When less stringent criteria (<30 mg/100 ml) are applied the incidence is at least double this (13 16) The incidence of hypoglycaemia is higher among small for date (SFD) infants In their recent study Lubchenco & Bard recorded blood glucose levels below 30 mg/100 ml in 67% of the pre-term 25% of the term and 18% of the post-term SFD infants in general obstetric service (13)

The purpose of this study is to report the incidence of hypoglycaemia among infants born to toxaemic mothers and to find the infants at greatest risk.

MATERIAL AND METHODS

All infants born to toxaemic mothers at the Department of Obstetrics and Gynaecology University of Oulu during one year were included in the study 2 49 mothers

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The reduced energy reserves in newborn infants of toxaemic mothers with severe pre-eclampsia and/or a low urinary oestriol level and in infants with intrauterine growth retardation, together with increased utilization of carbohydrates result in a high incidence of neonatal hypoglycaemia. An added stress, such as birth hypoxia and late initiation of feeding increase the likelihood of hypoglycaemia.

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All infants born to toxaemic mothers at the Department of Obstetrics and Gynaecology University of Oulu during one year were included in the study 2 49 mothers

Table 1 *Clinical data of mothers distributed by the severity of toxaemia*

Classification of toxaemia	No of mothers	Age (years)	Parity		Urinary oestriol excretion		Relative weight of placenta	
			Primi para	Multi para	<10 mg/24 h	≥10 mg/24 h	≤15%	>15%
Severe pre-eclampsia	51	29.4	24	27	16	24	11	43
Mild pre-eclampsia	73	27.7	33	40	7	46	13	64
Total	124	28.4	57	67	23	70	4	107

were delivered during this year and 133 of them had toxaemia (5.9%). The blood glucose levels of 9 infants born to toxaemic mothers were not followed thus the series is composed of the remaining 124 mothers and their 131 children (7 pairs of twins) Toxaemic mothers with latent or manifest diabetes mellitus were excluded, but mothers with hypertensio essentialis were included in so far as the toxaemia was considered to have been associated with the disease after the 24th gestational week

The mothers were divided into two groups according to the severity of toxaemia.

Mild pre eclampsia (73 mothers)

Blood pressure repeatedly ≥140/90 mmHg after the 24th gestational week and/or proteinuria repeatedly ≥0.1 g/24 h and/or weekly weight gain repeatedly ≥500 g, plus visible oedema in the face the abdomen and the extremities

Severe pre-eclampsia (51 mothers)

Blood pressure repeatedly ≥160/110 mmHg after the 24th gestational week and/or proteinuria ≥3.0 g/24 h and/or mild pre-eclampsia with subjective symptoms such as headache abdominal pain eye symptoms.

No eclampsia patients were included in the series. All the mothers were admitted to the Department of Obstetrics because of toxaemia during the last trimester of pregnancy The duration of the treatment before delivery varied from 4 to 45 days. The treatment of toxaemia consisted of bed rest and medication with diuretics (hydrochlortiazide furosemide or chlorthalidone) antihypertensive drugs (hydralazine chlonidine chloride or methyldopa) and sedatives (barbiturates or diazepam) in some cases. The duration of pregnancy ranged from 35 to 42 weeks. Caesarean section was necessary in 16 cases (13%)

Urinary oestriol excretion during 24 hours was assayed in 89 pregnancies including four twin pregnancies. Oestriol excretion was followed at [illegible]-day intervals during the treatment in hospital The number of oestriol assays per pregnancy varied from one to 22. The last daily urine was collected 1–4 days before delivery Oestriol excretion was considered low when the last oestriol excretion was <10 mg/24 h (23 patients)

The umbilical cord was clamped when pulsation ceased. The placenta with all its membranes was weighed and its relative weight

$$\left(\frac{\text{placental weight} \times 100}{\text{birth weight}}\right)$$

was calculated In 22 cases the relative weight of the placenta was 15% or less. The birthweight of 35 infants was below the 10th percentile in relation to the gestational age (22). Clinical data on the mothers and infants according to the severity of toxaemia are given in Tables 1 and 2

Blood glucose was recorded three times daily during the first 3 days of life If there was a single blood glucose value of <30 mg/100 ml the tests were continued for at least one day after the blood glucose level had returned to normal or after the therapy had been discontinued

At the time of the investigation breast feeding in our department was started routinely about 24 hours after birth During the first day the newborn received a 5% solution of saccharose orally If the blood glucose value

Table 2 *Clinical data of infants distributed by the severity of maternal toxaemia*

Classification of toxaemia	No of infants	Boys	Girls	Birth weight (g) arranged in percentiles			Apgar score 1 min		Apgar score 15 min		Gestational age (weeks)	
				<10%	10–90%	>90%	≤7	>7	≤7	>7	<36	36–42
Severe pre eclampsia	54	38	16	20	34	0	11	43	6	48	9	45
Mild pre eclampsia	77	42	35	15	55	7	11	66	3	74	[illegible]	75
Total	131	80	51	35	89	7	22	109	9	122	11	120

Table 3 *The occurrence of hypoglycaemia distributed by the severity of maternal toxaemia*

Severity of maternal toxaemia	Blood glucose values ≤20 mg/100 ml n	%		21–29 mg/100 ml n	%		≥30 mg/100 ml n	%
Severe pre-eclampsia (n = 54)	19	35	$p<0.01$	20	37 0	$p>0.30$	15	27.8
Mild pre-eclampsia (n = 77)	1	15 6		34	44.		31	40.2

was 20 mg/100 ml or less, intravenous administration of 10% glucose was started immediately regardless of whether the infants had symptoms or not. All the infants with symptomatic hypoglycaemia also received 10-20% glucose intravenously. 15 infants were given hydrocortisone as well

Blood glucose was determined from double capillary samples according to the glucose oxidase method (KABI) Daily urinary excretion of oestriol was determined by the method of Oakley (15)

The χ^2-test was used in the statistical analysis

RESULTS

The first blood glucose assay was made at an average age of 3 hours Thirty-one (23 7%) of the 131 infants had blood glucose values of 20 mg/100 ml or less. In addition 18 infants (13 7%) had two or more blood glucose values of 21–29 mg/100 ml and 36 infants (27.5%) had only one value of 21–29 mg/100 ml Altogether at least one blood glucose value below 30 mg/100 ml was found in 85 infants (64 9%) In this group of 85 infants who became hypoglycaemic the hypoglycaemia was diagnosed during the first 12 hours in 64 7% of the cases during the first 24 hours in 87 0% and after the second day in only 2.4%

The occurrence of hypoglycaemia according to the severity of the maternal toxaemia is presented in Table 3 Blood glucose values of 20 mg/100 ml or less were more frequent when the maternal toxaemia was severe than when it was mild. The difference is statistically significant

Blood glucose values of 20 mg/100 ml or less were most frequent (60 9%) among the infants born to toxaemic mothers with low urinary oestriol levels as can be seen from Table 4 All but five of the mothers in this group also had severe pre-eclampsia. Sixty five per cent (15/23) of these infants were SFD too Seventy per cent (16/23) of the infants born to mothers with low urinary oestriol levels had to be transferred to the pediatric department because of hypoglycaemia and/or respiratory difficulties The most severely ill newborns belonged to this group including 3 infants who died of respiratory difficulties during the first week of life

The occurrence of hypoglycaemia among the SFD infants and those with a normal birthweight is given in Table 5 Thirty-five (27%) of the 131 infants were SFD (below

ble 4 *The occurrence of hypoglycaemia distributed by the daily urinary excretion of oestriol*

ily urinary excretion of oestriol	Blood glucose values ≤20 mg/100 ml n	%		1–29 mg/100 ml n	%		≥30 mg/100 ml n	%
10 mg/24 h (n = 23)	14	60 9	$p<0.001$	4	17 4	$p<0.05$	5	21 7
10 mg/24 h (n = 70)	13	18.6		32	45 7		25	35 7

Table 5 *The occurrence of hypoglycaemia distributed according to small for date and normal birthweight infants*

	Blood glucose values							
	≤20 mg/100 ml			21–29 mg/100 ml			≥30 mg/100 ml	
Infant	*n*	%		*n*	%		*n*	%
Small for date (*n*=35)	13	37.[illegible]	$p<0.05$	12	34.4	$p>0.30$	10	28.6
Normal (*n*=96)	18	18.7		4[illegible]	43.8		36	37.5

the 10th percentile (22)). Blood glucose values of 20 mg/100 ml or less were more frequent in the SFD group than among the infants whose birthweight was in accordance with their gestational age. Of the 12 SFD infants whose mothers had severe pre-eclampsia and low daily urinary oestriol, nine (75%) had blood glucose levels below 20 mg/100 ml and only one had all its blood glucose values above 30 mg/100 ml.

The relative weight of the placenta did not correlate with the occurrence of hypoglycaemia.

Symptomatic hypoglycaemia (as symptoms were found such as tremor, cyanosis, paleness, limpness, irritability, apathy or tachypnoea, which disappeared during the treatment with glucose) was verified in 19 infants of the series (Table 6). Eight infants had symptomatic hypoglycaemia as the only disease and 11 had it in combination with other neonatal disorders, mostly birth asphyxia. Five other patients had severe respiratory difficulties concurrently with hypoglycaemia and it was therefore impossible to decide whether the symptoms were due to anoxia or hypoglycaemia.

DISCUSSION

Chronic placental insufficiency is one of the most typical features in the pathophysiology of toxaemia. Angiospasm not only in the placenta but also elsewhere leads to placental infarctions. This results in deterioration of the metabolism between the mother and the foetus as well as retardation of foetal growth. The stores of liver glycogen and fat in the foetus are inadequate. The newborn infant in such cases typically displays a

Table 6 *Distribution of 19 symptomatic hypoglycaemia patients according to the maternal toxaemia, oestriol excretion, birthweight and relative weight of placenta*

	Blood glucose values		
Patient groups	*≤20 mg/100 ml* No. of infants	*21–29 mg/100 ml* No. of infants	*≥30 mg/100 ml* No. of infants
Severe pre-eclampsia	10	3	0
Mild pre-eclampsia	6	0	0
Daily urinary excretion of oestriol			
<10 mg/24 h	14	3	0
≥10 mg/24 h	2	0	0
SFD infant	9	3	0
normal	7	0	0
Relative weight of placenta			
≤15%	3	2	0
>15%	13	1	0

high ratio of brain weight to liver weight an increased rate of oxygen consumption and excessive responsiveness to insulin (24 25) It is therefore difficult for the undernourished newborn of a toxaemic mother to maintain an adequate glucose level even without the influence of any additional factors partic ularly if the initiation of feeding is delayed If such an infant further experiences a lack of oxygen or other extra stress its need for energy increases and the possibility of hypoglycaemia becomes greater Our results show that hypoglycaemia is often associated with birth asphyxia and respiratory difficulties Only eight infants had symptomatic hypoglycaemia without any other disease

For several years maternal urinary excretion of oestriol has been regarded as the best criterion of placental insufficiency especially in toxaemia. The foetal adrenals and the placenta are needed to build up oestriol It has been noted in several studies that oestriol excretion correlates with neonatal morbidity and mortality as well as with birthweight and subsequent morbidity (6 8 11 26 27) Our study indicated that neonatal hypoglycaemia was significantly more frequent when maternal urinary oestriol was low than when it was normal Low urinary excretion of oestriol appeared to be a better prognostic criterion than either severe pre-eclampsia or low birthweight It was also shown that blood glucose levels of 20 mg/100 ml or less occurred significantly more often in the group with severe pre-eclampsia than in the group with mild pre-eclampsia, and also more often among the SFD infants than among those of normal birthweight, while relative weight of the placenta was unrelated to the occurrence of hypoglycaemia. This may be because in severe pre-eclampsia cases the birthweight decreases more than the weight of the placenta (14) The series included 12 SFD infants whose mothers had had severe pre-eclampsia and low urinary oestriol excretion nine of these twelve had blood glucose levels below 20 mg/100 ml In other words hypoglycaemia in these infants was rather the rule than an exception

Our series is based on a systematic screening of all the infants born to toxaemic mothers This probably explains why hypoglycaemia was noted as early as the first day of life in most of the cases The results agree with recent findings (1 19) but differ from those of earlier studies which are based on analyses of symptomatic newborn infants (2 5 10)

The frequency of hypoglycaemia in our series was very high Blood glucose levels of 20 mg/100 ml or less were recorded in 23 7% of all the infants and values below 30 mg/100 ml in 64 9% The incidences in earlier reports have been considerably lower Yet Lubchenco & Bard in a recent study in which they screened all newborns recorded blood glucose levels below 30 mg/100 ml in 67% of the pre-term SFD infants and 25% of the full-term SFD infants (13) The high incidence of hypoglycaemia in our series may be due to several factors such as late feeding and the severity of the toxaemia. At the time this series was collected feeding was not started until the age of 24 hours During the first day the infants were given only 5% saccharose Moreover cases of severe toxaemia are numerous in our hospital because toxaemic patients are admitted also from outside the normal area

Our actual purpose was to investigate the occurrence of hypoglycaemia in infants whose mothers had toxaemia of different degrees Since these infants often have concurrent respiratory difficulties, it was not always possible to distinguish between symptomatic and asymptomatic hypoglycaemia. Eight infants had symptomatic hypoglycaemia as their only disease while 11 had associated asphyxia. Five infants had severe respiratory difficulties concurrently with hypoglycaemia Although the administration of glucose did not clearly alleviate their symptoms it is possible that a part of them were due to hypoglycaemia. None of the symptomatic hypoglycaemic pa

tients had convulsions which are considered to be late symptoms of severe hypoglycaemia (12)

Sixteen of the 19 symptomatic hypoglycaemic patients had blood glucose values below 20 mg/100 ml while the remaining three only had several values of 21–29 mg/100 ml but *none of 20 mg/100 ml or less* Cornblath & Schwartz (5) proposed that a blood glucose value of 30 mg/100 ml could be taken as the criterion of hypoglycaemia for full-term infants and 20 mg/100 ml for SFD infants and pre term infants We found however that three SFD infants with blood glucose values of 21–29 mg/100 ml had symptomatic hypoglycaemia On the other hand none of the 36 infants with only one blood glucose value of 21–29 mg/100 ml and none of those with 30 mg/100 ml or more were found to have symptomatic hypoglycaemia It is evident that the duration of hypoglycaemia is a more important factor than a momentary lowering of the glucose level (12 18)

Since it became obvious that neonatal hypoglycaemia may cause central nervous system damage there has been a tendency in neonatal care to identify the infants potentially at risk and to prevent hypoglycaemia by such measures as early feeding and adequate oxygen treatment Infants born to toxaemic mothers have a great risk for hypoglycaemia. According to our results the infants whose mothers have had severe pre eclampsia and/or low excretion of urinary oestriol are at the greatest risk

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ENHANCEMENT OF FAT ELIMINATION DURING INTRAVENOUS FEEDING

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ABSTRACT Forget, P P F X Fernandes, J and Haverkamp Begemann P (Department of Paediatrics, Sophia Children s Hospital and Neonatal Unit, Erasmus University Rotterdam the Netherlands) Enhancement of fat utilization during prolonged intravenous feeding Acta Paediatr Scand 63 750 1974.—An 8-year-old girl with severe underweight caused by anorexia nervosa was treated with total intravenous nutrition for 4 weeks. During this period the Intralipid dose was stepwise increased the doses of Vamin and glucose were kept constant The Intralipid dose was monitored by the determination of the serum Intralipid levels. The fat utilization was investigated by intravenous fat tolerance tests and the estimation of postheparin lipoprotein lipase activity of the plasma The Intralipid elimination constant increased from 7% to 22%/min, the postheparin lipoprotein lipase activity increased from 50 to 317 μEq fatty acid/min/l These data enabled us to increase the Intralipid dose from 3 g fat/kg/per day to 8 g fat/kg/per day without an increase of the triglyceride blood levels. We may conclude that lipoprotein lipase is an inducible enzyme It is not clear which component of the hyper caloric intravenous regime causes this induction

KEY WORDS: Parenteral feeding postheparin lipoprotein lipase intravenous fat tolerance test

Little is known about the utilization and tolerance of fat emulsions in paediatric patients

An Intralipid (Vitrum) dose of 4 g/kg body weight per day has been used by some authors with acute side effects (6) and by others without any toxic effects (1) In the former studies the daily dose was infused over a period of 6 hours in the latter over a period of 24 hours It is tempting to infer that acute toxicity arises whenever the dose/time ratio is too high leading to high Intralipid blood levels

It has been shown by Hallberg (4) that fat tolerance tests with high dosage yield elimination curves which are biphasic At the point where one phase passes into the other lipoprotein lipase (LPL) the enzyme responsible for triglyceride elimination becomes saturated An Intralipid dose which exceeds the maximal LPL clearing capacity is probably slowly eliminated by aspecific ways such as phagocytosis by RES cells and liver (6) The plasma Intralipid levels must therefore not exceed a critical concentration of approximately 100 mg/100 ml when LPL becomes saturated

The Intralipid blood level depends on the one hand on the patient s clearing capacity for fat and on the other hand on the Intralipid dose

Recently we had the opportunity to study a patient who had to be fed parenterally for a prolonged period By determining this patient s clearing capacity at frequent intervals and by the daily investigation of Intra

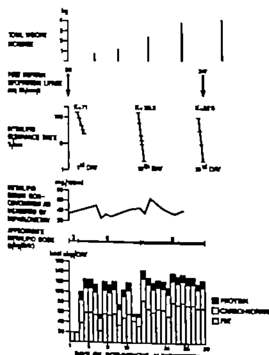


Fig. 1. Complete parenteral nutrition for 4 weeks in an 8-year-old child. The diagram shows the amount of fat (20% Intralipid), amino acids (Vamin) and glucose given. The changes in body weight, postheparin LPL, Intralipid clearance rate and Intralipid serum concentration are also recorded.

lipid blood levels we tried to get some more insight in the mechanism of fat clearing during parenteral feeding.

MATERIAL AND METHODS

The patient was an 8-year-old girl with a long history of anorexia. Her height was just under, her weight far below the third percentile. No evidence of organic disease was found. In close cooperation with our psychiatrists we decided to replace oral by parenteral feeding for 4 weeks.

We administered Intralipid 20% (Vitrum), Vamin (Vitrum) and glucose from separate bottles simultaneously at a constant rate around the clock. Caloric intake was stepped up from 60 to 120 calories/kg/day (Fig. 1). Vitamins and minerals were given orally.

The intravenous fat tolerance test (IVFTT) was carried out according to Carlson & Rössner (2). For all tests a 20% Intralipid emulsion was administered intravenously in a dose of 0.1 g fat/kg body weight, within one minute. Capillary blood samples were collected every 5 minutes for 30 minutes. The Intralipid concentrations were determined by nephelometry. For each disappearance curve a minimum of four consecutive points was used. The logarithms of the Intralipid concentrations were plotted against time. Straight lines were obtained from which the correlation coefficients were calculated. Steady state plasma Intralipid concentrations were estimated by nephelometry as well.

The postheparin LPL activities were measured 5 minutes after the intravenous injection of 100 U of heparin/kg body weight. The measurement of postheparin LPL activity has been described previously (5).

RESULTS

The Intralipid blood levels remained relatively constant during the whole period of intravenous feeding. Initial value for IVFTT was 7 1%/min. End value was 22.6%/min (Fig. 1). Correlation coefficients of the IVFTT curves were higher than 0.99 for each curve. Initial value for postheparin LPL was 50 μEq FA/min/l, end value 317 μEq FA/min/l (Fig. 1).

The triglyceride and cholesterol blood levels were determined before and after the intravenous feeding period and at the maximum Intralipid infusion rate. The triglyceride levels were always within the normal range, though slightly higher during the infusion period as compared with the prae and post infusion levels.

The cholesterol levels were slightly lower during the infusion period as compared with the prae and post infusion levels. The patient did not develop acetonuria, acidosis or coagulopathy and the liver function tests remained normal. The weight of the patient increased 4 kg in 4 weeks.

DISCUSSION

The concentration of Intralipid in the plasma at any given time during constant infusion represents a balance between the rate of entry into the plasma and the rate of removal. At a constant removal rate the Intralipid blood levels depend on the rate of entry into the blood. The observation in our patient that the plasma levels remained rela

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KEY WORDS Parenteral feeding postheparin lipoprotein lipase Intravenous fat tolerance test

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It has been shown by Hallberg (4) that fat tolerance tests with high dosage yield elimination curves which are biphasic At the point where one phase passes into the other lipoprotein lipase (LPL) the enzyme responsible for triglyceride elimination becomes saturated An Intralipid dose which exceeds the maximal LPL clearing capacity is probably slowly eliminated by aspecific ways such as phagocytosis by RES cells and liver (6) The plasma Intralipid levels must therefore not exceed a critical concentration of approximately 100 mg/100 ml when LPL becomes saturated

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POLYUNSATURATED FATTY ACID LIPIDOSIS INFANTILE FORM OF SO-CALLED NEURONAL CEROIDLIPOFUSCINOSIS

I *Clinical and Morphological Aspects*

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ABSTRACT Hagberg, B., Haltia, M., Sourander P., Svennerholm, L. and Eeg-Olofsson, O. (Departments of Paediatrics II Pathology I and Neurochemistry University of Gothenburg, Sweden and Pathology II, University of Helsinki, Finland). Polyunsaturated fatty acid lipidosis—an infantile form of so-called neuronal ceroidlipofuscinosis. Acta Paediatr Scand, 63:753 1974.—Clinical histological and ultrastructural findings in three children of Finnish origin and with a severe progressive encephalopathy are reported. The main symptoms were rapid developmental regression from about one year of age loss of speech, severe visual failure and pronounced secondary microcephaly A decerebrated state was reached within 1 3 years. Laboratory tests revealed a successively decreasing CSF γ-fraction The fatty acid composition of serum lecithin showed an increased amount of arachidonic acid in the early stage of the disease a pattern consistent with the biochemical changes in the brain.

The morphological characteristics consisted of extreme cerebral and cerebellar atrophy massive neuronal destruction associated with a pronounced macrophage and astrocytic reaction, and strongly PAS-reacting and autofluorescent granular deposits in the cytoplasm of the remaining cells. Electron microscopy revealed cytoplasmic osmiophilic deposits in the form of aggregates of globules with uniform and finely granular ultrastructure.

The condition was considered to be a nosological entity with a uniform clinical picture and characteristic ultrastructural changes in the brain. The evidence produced suggests that the disease described is identical with progressive heredodegenerative disorder known to have been diagnosed during recent years in more than 50 Finnish infants and small children and earlier described under the name of infantile type of so-called neuronal ceroidlipofuscinosis.

KEY WORDS Neurolipidosis, neuronal ceroidlipofuscinosis, fatty acid disorder amaurotic idiocy

1968 Hagberg, Sourander & Svennerholm published a preliminary report of a case late infantile progressive encephalopathy with pronounced morphological and biochemical changes in both the grey and white matter of the cerebrum and cerebellum. The brain tissue was extremely poor in lipids and the fatty acid analysis suggested a disturbed metabolism of the polyunsaturated fatty acids Combined clinical histological histochemical and biochemical investigations prompted the assumption of a previously unknown generalized metabolic disorder affecting particularly the brain and the retina We assumed that the new disease was very rare Recently however Haltia and co-

tively constant when the infused fat dose was brought up from 3 g to 8 g/kg body weight/day has to be explained by a rising rate of removal This has been confirmed in our patient by the increase in postheparin LPL activity parallel to the increased fat removal as measured by the IVFTT

It is not clear which component of the intravenous feeding regimen caused this enzyme induction Although no hormonal studies were performed in our patient hyperinsulinaemia has been observed during intravenous alimentation (3) Enhanced insulin production could be responsible for the anabolic response to parenteral feeding. Induction of LPL synthesis could be an aspect of this anabolic response

As for our patient the change of fat tolerance observed during parenteral feeding would have made the use of a standard fat infusion dose quite arbitrary By controlling lipid blood levels during the intravenous feeding period it was possible on the one hand to avoid hyperlipidaemia and on the other to take maximum benefit of the high caloric value of fat emulsions

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Table 1 *Clinical symptoms signs and tests in the present three cases compared to the Finnish series* (20)

	Average age in Finnish series 15 cases	Case 1 ♀ M L-	Case 2 ♂ T N	Case 3 ♀ A. N
Normal development 1st year of life	+(14/15)	+	+	+
Age at onset (years)	1–1½	1	1	1¼
Developmental stagnation/regression	1	1	1	1¼
Loss of speech/no speech appears	1 1¼	1¼	1¼	1¼
Severe dementia	1¼–1¾	1¼	1¾–2	1¾
"Knitting" hand stereotypies	1¾–2	?	?	2¼
Visual impairment	1¾	2	1¾	2¼
Blindness	1¾–4 (?)	3?	1¾–¾	Not yet at age 3
Myoclonic fits	1¾–2 (15/15)	1¾	3	¾
General convulsions	1¾–2¼ (10/15)	2¼	2	2¼
Microcephaly	++ (15/15)	++	++	+
Ataxic signs	1¼–1¾	1¾	?	1¼
Spastic signs	2–3	2¼	2	2¾
Retinal atrophy	1¾–3	(+)	(+?)	2¾
Fetal total flexion pattern	3–6	3–	3–4¼	Not yet at age 3
"Burnt-out" stage	2–3 (15/15)	2¼	2¼–3	Not yet at age 3
Death	5¼–6¼ (2/15)	5	¾	Still alive at age 3
Characteristic EEG-features	2–3 (15/15)	?	2	2¼
EMG and NCV	Normal		?	Normal
ERG extinguished	1¾–3 (14/15)	Not examined	Not examined	Not examined
CSF protein level	Normal	27 mg%	22 mg%	23–27 mg%
Decreasing CSF γ-fraction	+++	Not examined	+++	++
Vacuolated lymphocytes	–	–	–	–
Hypergranulation of neutrophils	±(5/15+)	?	?	–
Bone marrow	Normal (3/15)	?	?	Normal

3½ years. At 6 years the pattern was completely isoelectric.

X-ray PEG at 22 months revealed diffuse central and left-sided cortical atrophy

Biopsy A sural nerve biopsy specimen at 2¼ was normal (Sourander) No specimens were obtained of the rectum or brain in this case

Case 3

. N This 3-year-old girl, born on February 19 1970 the youngest sister of the child in case 2. Pregnancy delivery and the neonatal period were all uncomplicated. Birth weight 3060 g, length 50 cm, head circumference 33 cm She sat without support at 8 months, and started to walk at 14 months. At 14–16 months her motor performances first ceased to develop and then deteriorated, she stopped walking and became ataxic At the same time her speech—some single words—disappeared completely During the next half year she became demented and lost emotional contact with her mother But her vision seemed to be normal and at 21 months her fundi appeared normal Echo index was then 0.30 (normal) Grand mal seizures appeared at 2 / years She also had sudden myoclonic jerks in her right leg. At examination she was found to be ataxic and demented (imbecile). She drooled excessively and had stopped using her hands. She could creep and sit, but not stand or walk. Neurological examination revealed dyssynergia of cerebellar type and truncal tremor There were no signs of spasticity and her reflexes were all normal. Ophthalmological examination revealed nothing abnormal.

During the next half year (between 2 / and 2 / years of age) motor and mental state gradually regressed. She developed frequent hand stereotypies of a peculiar type. She now had positionally released muscular hypertonus in her legs, exaggerated muscle reflexes but no extensor plantar response Her vision was impaired but she was not blind Her fundi were now somewhat pale and slight optic atrophy could not be excluded. By 3 weeks of age she had deteriorated markedly Her dementia was severe. She was no longer able to sit, but could still roll over though with an obvious head lag She could no longer grip her hands She was still not blind but her vision was now severely impaired. Generalized dystrophic retinal changes were now seen. Spastic paraparesis had supervened.

Laboratory studies

Routine haematological and urinary tests revealed nothing remarkable. No vacuolated lymphocytes. Serum lipids at the age of 3 Cholesterol 1.60 phospholipids 1.82 and triglycerides 0.67 g/l.

The results of metabolic screening tests of urine including amino acid chromatography and determinations of organic acids, were normal.

CSF was first examined when the child was 1 /

workers (7 8 9 20) delineated an infantile type of so-called neuronal ceroid lipofuscinosis Their 18 Finnish patients had a disorder which in all essential clinical histological and histochemical respects resembled our case described in 1968 They have since seen a further 33 cases in Finland

Part I of this report gives a detailed account of the morphological findings in the original Swedish case (6) and of two further cases in siblings with the same clinical picture compares the clinical and morphological changes in these three cases with those in the Finnish series and summarizes our present knowledge of characterizing symptoms signs and tests for clinical suspicion in daily neuropediatric work The biochemical studies will be reported in a forthcoming paper (23)

CASE REPORTS

Case 1

M L. (reported in detail 1968 Hagberg et al. (6)). This girl born March 15 1961 was the only child of healthy non-related parents immigrants from Finland. Pregnancy delivery and development during her first year of life were uneventful At 12 months she was able to walk with support and to say several single words Stagnation of psychomotor development and regression of speech was first noted at the age of 12–15 months. At 18 months she was found to have ataxia and a skull circumference of 43 cm Three months later nodding spells of minor motor type appeared in series 20–30 times a day and her mental development regressed. Myoclonic jerks in arms and legs were also observed. At 2 years of age her vision seemed to deteriorate but the ocular fundi appeared normal Her skull circumference was now 43.5 cm Between 2 $^1/_4$ and 2 $^3/_4$ years of age her condition deteriorated rapidly to a soporous and very primitive motor state without voluntary movements. Spastic signs with ankle clonus and extensor plantar response had now appeared. But the ocular fundi still appeared normal She died at 5 years and 11 months of age in a decerebrated stage with continual fits Her eyes were never examined during her last years of life but she was obviously blind

Laboratory studies

Routine haematological and urinary tests revealed nothing remarkable No vacuolated lymphocytes Serum total lipids at the age of 2 were 7 4 cholesterol 2 75 phospholipids 2.58 and triglycerides 0.76 g/l Results of metabolic screening tests of urine including amino acid chromatography were normal Lumbar puncture revealed a normal cell count and protein content of 0.27 g/l The CSF was not examined electrophoretically

Case 2

T N This boy born May 22 1963 was the eldest of four siblings His youngest sister is described as case 3. The other two siblings are healthy as well as the probably non-related parents. Both parents come from the neighbourhood of Kalix near the Finnish border of Sweden The maternal grandmother came from Pietarsaari in a part of Finland where the disease in question seems to be particularly common (14) The paternal grandfather had been born in Finland too

Pregnancy was normal delivery occurred in the 38th week and was uncomplicated. Birth weight 2600 g. length 49 cm and skull circumference 31 cm. The boy was able to sit unsupported at 7 months and walked with support at 12 months, but he never learned to walk unsupported. He could say single words at 14–15 months. His vision was thought to be normal (?)

His first symptom was stagnation of his motor development particularly his gait from 12 months of age. At about 18 months his speech successively disappeared At 21 months his vision started to deteriorate markedly and he became blind within the next half year During the same period he stopped playing, regressed mentally and lost emotional contact with his mother Grand mal seizures started at 2 years. At 2 $^1/_2$ he could no longer sit unsupported. Between 2 $^1/_2$ and 3 years his condition deteriorated further to a final burnt out stage He was semisoporous without voluntary movements blind and severely demented.

Neurological examination at 22 months of age showed a retarded psychomotor development and a very obvious Babinski sign on the left At that stage his fundi were considered to be quite normal. At 2 $^1/_2$ years microcephaly (44 cm) dystonic tetraplegia with bilateral Babinski signs amaurosis and optic atrophy were noted. No other retinal changes were ever observed Bulbar signs appeared between 3 and 4 $^1/_2$ years. During the same period he also developed myoclonic jerks From that time on he was in a constant state of complete flexion hypertonia microcephalic (45 cm) and cachectic He died at 8 / years.

Laboratory studies

Routine examination of the blood and urine showed nothing remarkable No vacuolated lymphocytes Serum lipids at the age of 8 years were Cholesterol 1 62 and phospholipids 1 82 g/l. CSF protein was 0.22 g/l CSF electrophoresis showed almost complete absence of the τ fraction (at the age of 4 $/_2$) No metachromatic formations in urinary sediments Urinary sulphatases A and B were normal. No abnormal urinary excretion of acid mucopolysaccharide The results of metabolic screening tests of urine including amino acid chromatography were normal Normal tryptophan load test

EEG was first done when 2 years and revealed a diffuse abnormality with low amplitude delta and theta waves changes found to be further accentuated at

weighed only 305 g (Case 1) and 380 g (Case 2). The leptomeninges were thick and edematous. There was severe generalized gyral atrophy The cerebral cortex was only 1–1.5 mm thick and slightly yellowish grey It was difficult to distinguish from the under lying white matter which was greyish and severely reduced in amount It had a gela tinous appearance and was very tough The corpus callosum was no thicker than tissue paper and the ventricles were diffusely dilated. The basal ganglia and thalamus were shrunken. The cerebellum was small and firm and its folia were atrophic throughout The upper brain stem and pons showed con siderable reduction in size but the spinal cord was apparently less affected However its grey matter particularly the anterior horns, was conspicuously yellowish The cranial nerves (with the exception of the atrophic optic nerves) spinal roots and sciatic nerves exhibited no obvious macro-scopic alterations

Histological and histochemical findings

In the autopsy material (Cases 1 and 2) there was an almost total loss of nerve cells throughout the cerebral cortex The entire cortex consisted of a network of fibrillary hypertrophic astroglia with vary ing numbers of interspersed, frequently bi nucleate macrophages containing coarse granular deposits All the Purkinje cells and granule cells had disappeared from the cere bellar cortex Proliferated Bergmann's glia and a few macrophages formed a distinct layer at their previous site Most of the sub-cortical centres including the caudate nu cleus putamen, pallidum thalamus sub-stantia nigra pontine dentate and inferior olivary nuclei showed a severe or subtotal neuronal loss and were the site of massive astrocytosis and macrophage reaction. On the other hand most of the motor nerve cells of the brain stem motor anterior horn cells of the spinal cord and the spinal gang lion cells appeared to be preserved but showed considerable accumulation of gran ular deposits pushing the nucleus aside

The cerebral and cerebellar white matter was almost completely devoid of axons and myelin sheaths and showed diffuse astro-cytosis and astrogliosis In the brain stem and spinal cord the pyramidal tracts were largely degenerated but the posterior fas cicles and other ascending tracts appeared to be preserved No certain changes were found in the spinal roots or sciatic nerves In both autopsy cases the optic nerves were totally devoid of axons and myelin and con sisted mainly of collapsed sheaths of con-nective tissue with some remaining loose glial tissue in between

In Case 1 the eye was available for ex amination The photoreceptor cells of the retina had entirely degenerated and had been replaced by a number of pigment and lipid-laden macrophages Corresponding to the site of internal granular layer a large number of nuclei were visible most of them presum ably belonging to glial cells Almost all the neurons of the ganglion cell layer had been replaced by macrophages containing gran ular deposits

In the cerebral biopsy material from the frontal cortex (Case 3) the cortical cyto-architecture was severely disturbed. Many nerve cells were still left, but their cyto-plasm invariably contained coarse granular deposits and sometimes larger roundish in-clusions But no real ballooning was ob-served. Numerous diffusely distributed mac rophages constituted the most conspicuous cell type in the cerebral cortex (Fig. 1) They were frequently binucleate and con tained coarse granules (Fig. 1 inset) An additional remarkable feature was the tre mendous cortical fibrillary astrocytosis (Fig. 2) seemingly out of proportion to the actual tissue damage In the white substance there was slight reduction in the number of myelin sheaths and mild fibrillary astrocytosis The astrocytes and a few scattered macrophages

Table 2 *Fatty acid patterns of serum lecithin*

The control groups consisted of (A) patients with various neurological diseases (11) and (B) patients with ju
amaurotic idiocy

Fatty acid	Case 3 Å N			Case 2 T N	Mother	Controls			
						(A) (N=8) 32–67 yr		(B) (N=8) 8–16 yr	
	~6 yr	3 1 yr	3 3 yr	8.5 yr	29	Mean	S D	Mean	S
16 0	32	32	35	28	33	30.0	1.2	29 8	1 9
18 0	13	12	12	14	12	13.5	0.9	14 9	1
18 1	13	14	13	9 3	14	13.8	1 7	12.7	0 4
18 2 (n-6)	22	21	16	38	19	24 7	3 0	24 0	1 4
20 3 (n-6)	4 3	4 2	3 2	1 7	4 2	3 1	0 8	2.9	1 0
20 4 (n-6)	8.8	10	12	5 4	10	7 0	0.5	7 9	1 0
22 6 (n 3)	3 3	3 2	4 6	1 2	4 2	5 6	1 8	4 1	0 3

All values are expressed in molar percentages

years old The protein content was 0 23 g/l and electrophoresis revealed a moderately reduced γ-fraction A progressive reduction of the γ fraction was found when the patient was examined at the ages of ~ /₂ and 3 years

EEG At the age of 1 / a diffuse abnormality was revealed with low amplitude delta and theta waves At 2½ years the pattern was the same but accentuated and at 3 years the pattern showed almost isoelectric episodes

EMG was normal at 3 years

NCV The nerve conduction velocity rate in the peroneal nerve was 77 m/sec

X ray PEG revealed pronounced diffuse cortical atrophy

Biopsy Brain and liver biopsies were performed in September 1972 The findings are given below

The main clinical features of the three patients are summarized in Table 1

Serum Fatty Acid Analyses

Determinations of the fatty acid patterns of serum lecithins and triglycerides from patients 2 and 3 and their mother were performed with gas liquid chromatography after separation on thin layer plates as recently described by Karlsson et al. (11) The values are compared with those of two control series one consisting of patients with various neurological diseases the other of patients with juvenile amaurotic idiocy (Spielmeyer Sjögren) It is obvious from Table 2 that in patient 3 and the mother the serum lecithins had a low linoleic acid concentration while the arachidonic concentration was raised. Otherwise the values did not differ significantly from those in the control groups. In Case 2 T N the fatty acid composition of serum lecithin was extreme with a very high linoleic acid level and low oleic and arachidonic acid levels. The fatty acid composition of serum triglycerides was normal in the mother while in patient 3 linoleic acid was increased Case 2 showed an extremely high value fo linoleic acid 44 molar percentages compared with 13. and 12.6 molar percentages in the two control groups.

Morphologic and Histochemical Studies

Material and methods

The autopsies in Cases 1 and 2 were performed within 24 hours of death The central nervous system was removed and the left cerebral hemisphere of these patients, part of the cerebral biopsy material from the right frontal lobe of Case 3 specimens from internal organs and samples from the sciatic nerves of the autopsy cases were fixed in 10% neutral formalin solution Paraffin sections from representative blocks were stained with haemotoxylin eosin luxol fast blue-cresyl violet PAS (McManus) Sudan black B and long Ziehl-Neelsen methods Unstained sections were studied in ultraviolet light (HBO 200 mercury lamp UG 1 (4 m energy filter K 430 barrier filter) Frozen sections we stained with the PAS (McManus) Sudan black B sca let red, OTAN and von Hirsch-Peiffer methods as we as with Cajal s method for astrocytes.

For electron microscopy tiny pieces were cut fro the fresh cerebral biopsy material obtained in Case (frontal cortex and subcortical white matter) and fro formalin fixed autopsy material in Cases 1 and 2 (fronta cortex and white matter anterior horns of the spinal cord) The specimens were fixed in a cacodylate-buf fered mixture of glutaraldehyde and paraformaldehyde at pH 7 2 (12) post-fixed in osmium tetroxide dehydrated in a graded ethanol series and embedded in Epon 812. Thin sections were cut on a LKB Ultrotome III stained with uranyl acetate and lead citrate and examined under a Hitachi HS-7S electron microscope.

Macroscopic findings

At post mortem examination of Cases 1 and 2 the brain was exceedingly small and

weighed only 305 g (Case 1) and 380 g (Case 2). The leptomeninges were thick and edematous. There was severe generalized gyral atrophy. The cerebral cortex was only 1-1.5 mm thick and slightly yellowish grey. It was difficult to distinguish from the underlying white matter, which was greyish and severely reduced in amount. It had a gelatinous appearance and was very tough. The corpus callosum was no thicker than tissue paper and the ventricles were diffusely dilated. The basal ganglia and thalamus were shrunken. The cerebellum was small and firm, and its folia were atrophic throughout. The upper brain stem and pons showed considerable reduction in size, but the spinal cord was apparently less affected. However, its grey matter, particularly the anterior horns, was conspicuously yellowish. The cranial nerves (with the exception of the atrophic optic nerves), spinal roots, and sciatic nerves exhibited no obvious macroscopic alterations.

Histological and histochemical findings

In the autopsy material (Cases 1 and 2) there was an almost total loss of nerve cells throughout the cerebral cortex. The entire cortex consisted of a network of fibrillary hypertrophic astroglia with varying numbers of interspersed, frequently binucleate macrophages containing coarse granular deposits. All the Purkinje cells and granule cells had disappeared from the cerebellar cortex. Proliferated Bergmann's glia and a few macrophages formed a distinct layer at their previous site. Most of the subcortical centres, including the caudate nucleus, putamen, pallidum, thalamus, substantia nigra, pontine, dentate, and inferior olivary nuclei, showed a severe or subtotal neuronal loss and were the site of massive astrocytosis and macrophage reaction. On the other hand, most of the motor nerve cells of the brain stem, motor anterior horn cells of the spinal cord and the spinal ganglion cells appeared to be preserved, but showed considerable accumulation of granular deposits pushing the nucleus aside.

The cerebral and cerebellar white matter was almost completely devoid of axons and myelin sheaths and showed diffuse astrocytosis and astrogliosis. In the brain stem and spinal cord the pyramidal tracts were largely degenerated, but the posterior fascicles and other ascending tracts appeared to be preserved. No certain changes were found in the spinal roots or sciatic nerves. In both autopsy cases the optic nerves were totally devoid of axons and myelin and consisted mainly of collapsed sheaths of connective tissue with some remaining loose glial tissue in between.

In Case 1 the eye was available for examination. The photoreceptor cells of the retina had entirely degenerated and had been replaced by a number of pigment- and lipid-laden macrophages. Corresponding to the site of internal granular layer a large number of nuclei were visible, most of them presumably belonging to glial cells. Almost all the neurons of the ganglion cell layer had been replaced by macrophages containing granular deposits.

In the cerebral biopsy material from the frontal cortex (Case 3) the cortical cytoarchitecture was severely disturbed. Many nerve cells were still left, but their cytoplasm invariably contained coarse granular deposits and sometimes larger roundish inclusions. But no real ballooning was observed. Numerous diffusely distributed macrophages constituted the most conspicuous cell type in the cerebral cortex (Fig. 1). They were frequently binucleate and contained coarse granules (Fig. 1, inset). An additional remarkable feature was the tremendous cortical fibrillary astrocytosis (Fig. 2), seemingly out of proportion to the actual tissue damage. In the white substance there was slight reduction in the number of myelin sheaths and mild fibrillary astrocytosis. The astrocytes and a few scattered macrophages

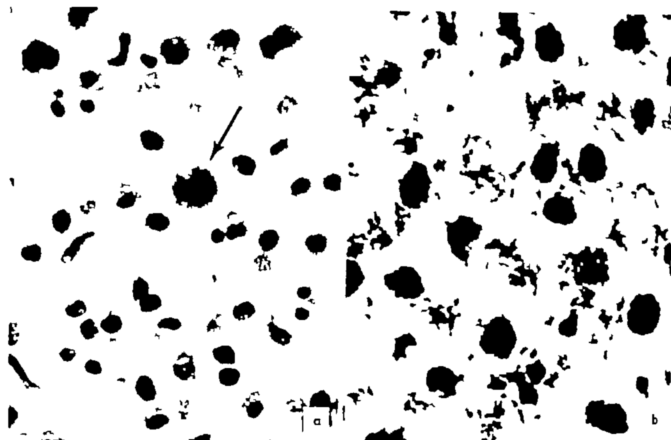


Fig 1 (*a*) A typical area of the frontal cortex (brain biopsy material) Note the numerous macrophages one of which is binucleate (*arrow*) No nerve cells are visible in this field Paraffin section haematoxylin eosin high power (*b*) Corresponding area. Note the intensive sudanophilia of the macrophages. Between them hazy astrocytes with sudanophilic granules are seen Paraffin section Sudan black B high power

contained granular deposits similar to those in the nerve cells

The granular deposits observed in all cases in the nerve cells macrophages and astrocytes were autofluorescent They reacted positively with Sudan black B and PAS (McManus) and were acid fast and resistant to lipid solvents

Electron microscopic observations

The long terminal stage of the disease autolysis and inadequate fixation did not appear to have any marked effect on the ultrastructure of the abnormal cytoplasmic deposits because essentially similar osmiophilic inclusions were observed in the autopsy material (Cases 1 and 2) and in the brain biopsy (Case 3) particularly in the nerve cells macrophages and astrocytes These inclusions consisted of large usually membrane bound aggregates of small spherical globules 0 2–0 4 μm in diameter The internal structure of these globules was quite uniform and finely granular No curvilinear bodies or cytosomes with fingerprint patterns were seen Occasional zebra like bodies were encountered particularly in the biopsy case

DISCUSSION

The morphological changes in all three Swedish cases were essentially the same although the alterations were much less advanced in the biopsy specimen of the brain (Case 3 Å N) than in the autopsy cases The autopsy cases showed an extreme brain atrophy with an almost complete loss of cerebral and cerebellar cortical neurons and massive destruction of the basal ganglia and thalamus The neuronal destruction—parti

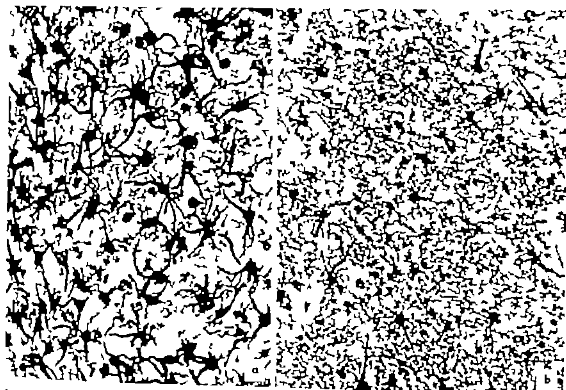


Fig. 2 a and b. Frontal cortex (*a*) and subcortical white matter (*b*) (brain biopsy material). Note the pronounced astrocytic hyperplasia and hypertrophy in the cerebral cortex. Frozen section, Cajal, medium power

cularly in the early biopsy case—was associated with a massive macrophage and astrocytic reaction in the grey matter. At autopsy of the cases of long duration this impressive mesenchymal and glial reaction appeared to have largely subsided in the cerebral cortex, but to have continued in subcortical centres, where active neuronal destruction was still in progress. The remaining neurons, macrophages, and astrocytes in the autopsy cases as well as in the biopsy specimen showed characteristic osmiophilic deposits of granular type in their cytoplasm. In the white matter there was an almost complete loss of myelin sheaths in the autopsy cases. All these features are in good agreement with the observations in the Finnish cases (8, 9).

For differentiation from other similar degenerative encephalopathies during the first years of life, the electron microscopic picture—cytoplasmic osmiophilic deposits in the form of aggregates of globules with a uniform and finely granular ultrastructure—is important. No cytosomes with curvilinear structures or fingerprint-type pattern, characteristic of the various forms of the Batten-Vogt syndrome (24), were encountered. The occurrence in our cases of zebra-like bodies, first described in the cerebral neurons of patients with the Hurler-Hunter syndromes (1) and later in some cases of the Batten-Vogt syndrome (2, 3, 4, 5, 10), for example, is probably a non-specific phenomenon.

Our 3 patients showed a uniform and characteristic clinical picture, closely resembling to that reported by Santavuori et al. (20) in 15 Finnish children (Table 1). The combined clinical, histological and ultra-

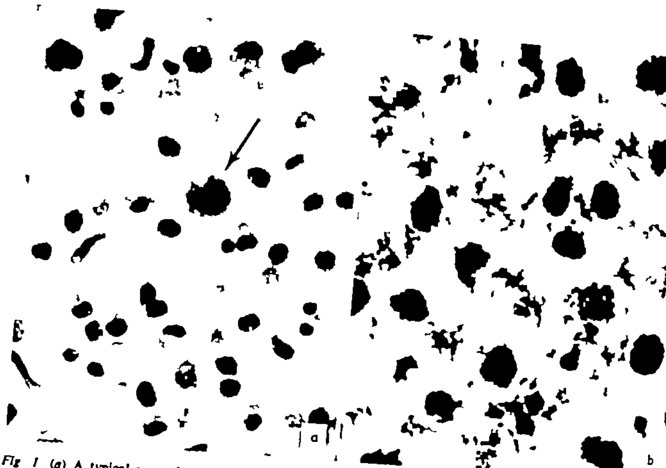


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Fig. 4. Cytoplasmic deposits from a cortical neuron (from biopsy material from case 3, a girl aged 2 years 7 months). Except for less marked lobulation the granular neuronal inclusions between the cisternae of the rough-surfaced endoplasmic reticulum are essentially similar to those in the autopsy specimen of the brother (Fig. 3). Occasional zebra-like bodies are encountered (*inset*). ×62 000

developmental stagnation and regression, the few words of speech completely disappear, the emotional contact with the surroundings ceases, "knitting" hand stereotypies appear, impairment of vision becomes more and more obvious. Myoclonic jerks regularly occur. Within 1–3 years the children are in a decerebrated semi-soporous burnt out state. The patients are microcephalic. The first neurological abnormalities noticed are signs of ataxia and hypotonus, followed by spastic signs starting in the lower limbs. Retinal dystrophy with generalized hypopigmentation of the entire fundus successively develops (17, 20), but may be difficult to recognize until relatively late. The final stage exhibits a picture of complete flexion, hypertonus and blindness.

The EEG and ERG abnormalities are unspecific but of considerable help for differentiating the disorder from other diseases, particularly the late infantile type

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of so-called neuronal ceroidlipofuscinosis (Jánsky Bielschowsky) The EEG rapidly changes from normal to almost isoelectric within 1 1/2–3 years (19 20) photic stimulation produces no polyphasic spikes consistently observed in the Jánsky Bielschowsky disease (13 15 16) The ERG is extinguished at an early stage even when the ophthalmoscopic findings are insignificant (20)

Although the clinical pattern and course are characteristic and suggestive the clinical picture has not revealed any pathognomic symptom or sign permitting an easy and reliable diagnosis during life Routine laboratory tests of the blood urine and CSF were non-informative No vacuolated lymphocytes were observed Of special laboratory examinations CSF electrophoresis in our last two cases revealed successive diminution and final disappearance of the τ fraction τ is formed by the hydrolysis *in vivo* of transferrin by endogenous sialidase The brain sialidase is enriched in the synaptosomal fraction (26) and a severe diminution of the sialidase activity is well correlated with the almost total neuronal loss found in biopsy specimens of the brain or at autopsy

So far the biochemical studies have not revealed the primary enzymatic lesion or the chemical composition of the stored material by which the new disease can be specifically diagnosed The fatty acid composition of serum lecithin in Case 3 however showed some characteristic features (Table 2) Arachidonic acid 20 4 (*n*-6) and its immediate precursor 20 3 (*n*-6) were increased while dokosahexaenoic acid 22 6 (*n* 3) was decreased This is the same pattern as that found for the fatty acid changes in brain phosphoglycerides (6 23) Determination of the fatty acid composition in serum lecithins from other cases and the obligatory heterozygotes will show whether we by gas chromatographic analysis of the fatty acid profile of serum lecithin have found a method for the early diagnosis of this disease during life and possibly for the detection of carriers

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Table 1 *The mean creatinine coefficient (creatinine per kg body weight per 24 hours urine excretion) during the first eight days of life*

Days after birth	1	2	3	4	5	6	7	8
Creatinine coefficient, mg/kg/24 hrs	4.0	4.6	7.9	8.7	8.6	8.7	8.0	7.1

After being thawed, a small sample was removed from each urine for creatinine determination (12). Using the mean normal body weight and the average daily urine volumes (16), the creatinine coefficient (mg/kg body weight/24 hours) was calculated

The urines were dialyzed, lyophilized and passed through a Sephadex G 100 column as earlier described (9). The fractions containing the ESF and the EIF were collected and lyophilized. Before testing, the fractions were dissolved in normal saline giving an amount corresponding to 5 mg creatinine per ml

The exhypoxic polycythemic mouse bioassay was used for ESF and EIF determinations as described in detail elsewhere (8). At least five mice were used in each assay group, in the control groups even ten mice. In all assays, 1 ml test material was injected. Mice with PCV below 55 vol% at the end of the study were discarded.

The stimulatory effect of the ESF fractions were measured as the per cent ^{59}Fe incorporation into newly formed red blood cells. The inhibitory effect of the EIF fractions were measured as the decrease in the ^{59}Fe incorporation in the mice receiving EIF compared to the control mice. The control groups received an external ESF-standard and saline while the mice in the experimental groups received the ESF-standard and the EIF fraction, injected simultaneously. The ESF injections were given subcutaneously while the EIF was given intraperitoneally. The difference in the ^{59}Fe uptake between the two groups, represents the degree of inhibition. The individual per cent inhibition in each of the four test assays were also calculated and expressed as the average from each day

RESULTS

Table 1 shows the mean creatinine coefficient in the different age groups. From the third day of life a constant excretion of about 8 mg creatinine/kg/24 hours is obtained

Table 2 shows the stimulatory effect of the ESF fractions from the first 8 days of life after removal of the EIF. A demonstrable amount of ESF is only found in the urine voided the first day of life

In Table 3 the inhibitory effect of the EIF fractions from the first week of life is presented. The results are expressed as the per cent ^{59}Fe incorporation compared with the ESF stimulated control groups from the four individual assays. In Fig. 1 however the mean per cent inhibition from each day of life is presented. From the third day of life a more marked constant inhibitory effect is obtained. However an inhibitory effect of the EIF preparations from the first day is also present. The values are significantly different from the control levels and the values from day 6–8 are also significantly lower than the value from day 2 ($p<0.05$)

DISCUSSION

At term raised ESF levels are found in cord blood (4) and in the first voided urine (1). However no ESF can be detected in blood

Table 2 *The stimulatory effect of the ESF fractions in urine from the first 8 days of life after removal of EIF expressed as the per cent ^{59}Fe incorporation into red blood cells*

Test material	% ^{59}Fe uptake ± S.E. (No. of mice)
NaCl	0.45±0.06 (11)
ESF	
Day 1	2.05±1.08 (9)
Day 2	0.37±0.05 (14)
Day 3	0.43±0.06 (15)
Day 4	0.40±0.06 (13)
Day 5	0.43±0.07 (14)
Day 6	0.35±0.05 (15)
Day 7	0.37±0.06 (10)
Day 8	0.40±0.13 (8)

URINARY EXCRETION OF ERYTHROPOIETIN AND ERYTHROPOIESIS INHIBITORS IN THE NEONATAL PERIOD

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ABSTRACT Lindemann R. (Pediatric Research Institute Department of Pediatrics, University Hospital Rikshospitalet Oslo Norway). Urinary excretion of erythropoietin and erythropoiesis inhibitors in the neonatal period Acta Paediatr Scand 63 764 1974. —The regulation and suppression of erythropoiesis in the neonatal period has been studied Urines from normal newborn babies were collected from time of delivery and during the first week of life Both erythropoietin (ESF) and an erythropoiesis inhibiting factor (EIF) were separated by Sephadex gel filtration The factors were tested according to days after birth and related to the creatinine coefficient. The exhypoxic polycythemic mouse bioassay was used and the results were expressed as the per cent incorporation of ^{59}Fe into newly formed red blood cells. A demonstrable amount of ESF was found only in the urine voided the first day of life indicating that the ESF production is shut off immediately after birth. From the third day of life a marked inhibitory effect of the EIF fractions was found. This may indicate a dual mechanism behind the decrease in neonatal erythropoiesis: a shut off of the ESF production and the appearence of erythropoiesis inhibitors.

KEY WORDS: Erythropoiesis, eryhtropoietin, erythropoiesis inhibiting factor neonatal period urine

During the first week of extrauterine life a marked decrease in the erythropoietic activity occurs Erythropoietin (ESF) levels are slightly elevated in cord blood (4) while ESF is not detectable neither in serum (4 10) nor in urine (9) collected after the second day in the neonatal period

Eryhtropoietic inhibitors have been found in plasma from normal newborn babies from the fourth day of life (15) However some investigators have failed to demonstrate these inhibitors (11) The difference in these findings may be due to variations in techniques

An erythropoiesis inhibiting factor (EIF) has also been found in urine from normal healthy individuals (7 8) and normal infants (9)

The question arises if the lack of erythropoietic stimulatory effect of the serum and urine from newborn infants is due to increased amounts of inhibitors decreased amounts of stimulators or both? The present study was undertaken in order to try to investigate the role of ESF and EIF on erythropoiesis during the first week of life by separating and quantitating the daily excretion of these two factors in urine

MATERIAL AND METHODS

Urine was collected from normal newborn babies from time of delivery until 8 days of life In order to get satisfactory amounts urines were pooled from several babies in groups according to age. Urine voided between 0 and 4 hours after delivery was designated "day 1" between 24 and 48 hours "day 2" etc. Urines were collected during four different periods. During each period the urinary specimens were pooled according to age and frozen at −20°C immediately after voided. Four samples were thus available for testing from each day

Using urine and the procedure of Sephadex gel filtration, the two factors ESF and EIF can be separated and studied individually (8). This may give a more precise answer to the question of erythropoietic suppression postnatally. The first part of this paper demonstrates a lack of ESF in the urine from the second day of life. The presence of some ESF in the urine voided the first day is in accordance with the findings of Finne (1). The great variance in the results from the first day (Table 2) could be due to different amounts of the first voided urine in the four urine specimens and the time of voiding perinatally.

In order to quantitate the daily excretion of ESF and EIF a reference parameter had to be chosen. In adult life the amounts excreted per 24 hours is used as a quantitative parameter. A 24-hour urine sample is difficult to achieve in a large number of newborn infants. In this study pooled urine samples were used. The unstable kidney function and variable urine volumes, particularly the first days of life (16), had also to be taken into consideration. These factors would therefore induce an error in the quantitation. To overcome this problem the creatinine coefficient was chosen as parameter, since it seems constant from the third day of life (Table 1). This finding is supported by studies on newborn lambs which show an adult type of renogram from the third day onwards, indicating the foetal kidneys to be dormant the first days of life (5).

The second part of this paper presents data on the inhibitory effect of the EIF fractions in urine during the first week of life. A more marked inhibitory effect is found from the third day (Table 3, Fig. 1). This is in accordance with the findings of erythropoiesis inhibitors in plasma which occurred from the fourth day of life (15). The inhibitory effect, observed in the EIF fraction from day 1" could thus be due to the variable reference parameter the first day of life.

This may indicate that a second mechanism, an inhibition o erythropoiesis takes an active part in the regulation of erythropoiesis in the neonatal period.

Variations in one or both of these two factors could therefore result in a change in the red cell mass. Neonatal polyglobulia, i.e. a venous haematocrit above 70 vol%, could be obtained in different ways. Either as a materno-foetal transfusion or as the result of increased production of ESF as seen in patients with placental dysfunction or other conditions giving an intrauterine hypoxia (2). On the other hand, a delay in the appearance of inhibitors could also produce a lack in the erythropoietic suppression postnatally. This possibility has been demonstrated in some patients with the syndrom neonatal polyglobulia (9), but more data are needed to confirm these findings.

The present data give evidence of two mechanisms taking part in the suppression and regulation of erythropoiesis after birth. An absolute shut off of the ESF production and the appearance of erythropoiesis inhibitors.

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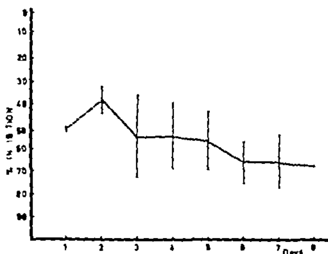


Fig 1 The mean per cent inhibitory effect of the EIF fractions in urine during the first 8 days of life expressed as per cent of the individual observations.

from the second day of life or during the following 6 to 8 weeks (4 10) This indicates a marked decrease in the erythropoietic stimulatory activity during the postnatal period Studies on bone marrow (6) reticulocytes (14) and iron kinetics (3) all support this These findings are compatible with the concept that the suppression of erythropoiesis is due to improved oxygenation of the oxygen sensitive cells in the kidney resulting in cessation of ESF production

From the fourth day of life the appearance of erythropoiesis inhibitors have been demonstrated in plasma obtained from normal new born babies (15) indicating a second regulatory mechanism taking part in the postnatally suppression of erythropoiesis Some investigators however have failed to demonstrate these inhibitors in newborn serum (11) The assay method is of great importance for demonstration of an inhibitory effect The EIF has to be injected simultaneously with or some hours before the ESF stimulation in order to obtain a depression of the erythropoietic stimulus (unpublished data) Another time schedule was used by Matoth & Zaizov in their studies (11)

Studies in high altitude dwellers brought down to sea level a condition similar to the period immediately after birth have also documented the appearance of erythropoiesis inhibitors in plasma (13) during periods with a surplus of circulating haemoglobin.

Table 3 *The inhibitory effect of the EIF fractions in urine the first 8 days of life expressed as the per cent ^{59}Fe incorporation into red blood cells*

	% ^{59}Fe uptake ± S E. (No of mice)			
Test material	Assay 1	Assay 2	Assay 3	Assay 4
NaCl	0 39±0.11 (5)	0 66±0 05 (3)	0 57±0 01 (3)	0.47±0 08 (4)
NaCl + ESF	3 24±0 61 (9)	5 61±0.73 (5)	7 31±0 89 (10)	8 3?±1 03 (7)
EIF				
Day 1 + ESF	1.54±0 66 (5)	–	3 68±0 45 (4)	4 09±0.85 (5)
Day 2 + ESF	0.30±0.07 (5)	4 13±1 20 (4)	4 03±1 00 (4)	4 75±0.54 (5)
Day 3 + ESF	0.50±0.18 (5)	3 64±0.36 (5)	0 62±0 05 (5)	5 16±1 20 (5)
Day 4 + ESF	1.83±0 67 (5)	? 98±0.56 (5)	1 13±0.57 (5)	5.58±0 64 (4)
Day 5 + ESF	0.34±0 18 (4)	2.35±0.35 (4)	1 62±0.37 (4)	5 49±0 69 (5)
Day 6 + ESF	0.38±0.10 (5)	2 29±0 26 (5)	2 02±0.17 (5)	4 77±1 45 (5)
Day 7 + ESF	0 41±0.07 (5)	2.82±0.52 (5)	–	3 33±1 31 (4)
Day 8 + ESF	0 99±0.39 (5)	–	–	–

Using urine and the procedure of Sephadex gel filtration, the two factors ESF and EIF can be separated and studied individually (8). This may give a more precise answer to the question of erythropoietic suppression postnatally. The first part of this paper demonstrates a lack of ESF in the urine from the second day of life. The presence of some ESF in the urine voided the first day is in accordance with the findings of Finne (1). The great variance in the results from the first day (Table 2) could be due to different amounts of the first voided urine in the four urine specimens and the time of voiding perinatally.

In order to quantitate the daily excretion of ESF and EIF a reference parameter had to be chosen. In adult life the amounts excreted per 24 hours is used as a quantitative parameter. A 24-hour urine sample is difficult to achieve in a large number of newborn infants. In this study pooled urine samples were used. The unstable kidney function and variable urine volumes particularly the first days of life (16) had also to be taken into consideration. These factors would therefore induce an error in the quantitation. To overcome this problem, the creatinine coefficient was chosen as parameter since it seems constant from the third day of life (Table 1). This finding is supported by studies on newborn lambs which show an adult type of renogram from the third day onwards indicating the foetal kidneys to be dormant the first days of life (5).

The second part of this paper presents data on the inhibitory effect of the EIF fractions in urine during the first week of life. A more marked inhibitory effect is found from the third day (Table 3, Fig. 1). This is in accordance with the findings of erythropoiesis inhibitors in plasma which occurred from the fourth day of life (15). The inhibitory effect, observed in the EIF fraction from day 1, could thus be due to the variable reference parameter the first day of life.

This may indicate that a second mechanism, inhibition of erythropoiesis takes an active part in the regulation of erythropoiesis in the neonatal period.

Variations in one or both of these two factors could therefore result in a change in the red cell mass. Neonatal polyglobulia, i.e. a venous haematocrit above 70 vol% could be obtained in different ways. Either as a materno-foetal transfusion or as the result of increased production of ESF as seen in patients with placental dysfunction or other conditions giving an intrauterine hypoxia (2). On the other hand a delay in the appearance of inhibitors could also produce a lack in the erythropoietic suppression postnatally. This possibility has been demonstrated in some patients with the syndrom neonatal polyglobulia (9) but more data are needed to confirm these findings.

The present data give evidence of two mechanisms taking part in the suppression and regulation of erythropoiesis after birth. An absolute shut off of the ESF production and the appearance of erythropoiesis inhibitors.

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THE NEPHROTIC SYNDROME OF INFANCY

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ABSTRACT Bouton J. M., and Coulter J. B. S. (Departments of Pathology and Child Health, Alder Hey Children's Hospital Liverpool England) The nephrotic syndrome of infancy Acta Paediatr Scand, 63:769 1974.—Nine cases of the nephrotic syndrome occurring in infancy in the years 1954–70 are presented Clinical, laboratory and histological data are documented. Although they do not form a homogeneous group an effort is made to group them particularly as to their resemblance to the congenital nephrotic syndrome as found in Finland. The histology of microglomeruli and ectasia of the renal tubules, though distinctive, can be seen in other renal diseases. The mechanism of virtually inevitable death and the suddenness of death is discussed and suggestions are made as to supportive therapy particularly regular gamma globulin injections, for prophylaxis against infection.

KEY WORDS: Nephrotic syndrome, infancy

The nephrotic syndrome occurring in infants under one year is rare (2 3 43) Sometimes a predisposing factor can be incriminated such as syphilis (30 38) mercury (2 47) cytomegalic inclusion disease (7) or part of the hereditary onycho-osteodysplasia syndrome (Hood) (41) Renal vein thrombosis although a cause of the nephrotic syndrome in adults seems rarely to be a primary event in infants (1) usually being found as a secondary phenomenon (32) The majority of cases have been described from Finland (17 18) where an autosomal recessive inheritance (32) with 103 cases in 80 families (18) has been found and the name congenital nephrotic syndrome (CN) (16) has been adopted.

A small number of cases of the CN have been described from each of a large number of countries other than Finland particularly Great Britain (12 31 44) France (13) and other parts of Europe and North America (47) also the Far East in Japan (24) and in the Maori race in New Zealand (23) Interestingly 3 out of 4 infants described by Hoyer et al (21) from Minnesota were of Finnish extraction The distribution of cases has been reviewed by Norio (32) and more recently by Hallman et al (18) and extensively documentated by the Finnish workers

The CN as it occurs in Finland has a fairly uniform pattern The infants are often slightly pre-term and of relatively low birth weight for the gestational age The placenta is almost always larger than normal being over one-quarter of the birth weight of the baby (25) Proteinuria and oedema may be present at birth and was present in 94% by 8 weeks of age (32) The serum proteins have a nephrotic pattern (37) and the cholesterol often only slightly raised initially reaches high levels later on. Serum IgM is typically raised

Histologically the chief features are ectasia of the proximal convoluted tubules and

roliferative changes in the glomeruli which ter may become extensively hyalinised owman's capsule may be distended By lectron microscopy the common findings re fusion of the foot processes of the pithelial cells and nodular thickening of the asement membrane

It is our intention to review and analyse e available data of 9 cases of the nephrotic yndrome occurring in infancy that were een in the period 1954–72 at the two chil ren's hospitals in Liverpool which serve population of 1 ½ million.

Table 3 *Urinary findings*

Case	RBC	WBC	Casts	Infec tion	Aminoacid chromatography
1	+	–	–		Not done
2	+	+	–		Moderate generalised increase
3	–	+	+	+	Normal
4	++	+	+	+	Not done
5	+	++	+	+	Not done
6	Scanty	Scanty	+	–	Mild generalised increase
7	++	+	–		Normal
8	–	–		+	Not done
9	+	Scanty	+	+	Normal

Clinical data

he onset of oedema was by 10 weeks in 8 patients Table 1). Case 5 C. H., age of onset 9 months, is cluded because of the similarity of the renal histology other cases of the CN C. H. was born in Lusaka, orthern Rhodesia to parents of English origin who had me to Rhodesia from Liverpool.

Only one infant (Case 6) was definitely pre-term. hree infants (Cases 1 2 and 9) were light for gestanal age according to the data of Thomson et al. 3) Unfortunately the placenta weight is known in only cases (6 and 9) but in both the placenta weight is early half the birth weight. Generally speaking, the ood pressure was normal. A slightly raised blood essure was seen in Cases 6 and 9 130/75 and 125/80 spectively Palpably enlarged kidneys were found in ses 7 and 9 Patient 4 had a right inguinal hernia and

...mia ...ng ...cemia	Treatment
...alytic. ...p ...ds	Nil
	Cortisone Antibiotics
...h	Nil
...ococci	Prednisolone Antibiotics
...ds	Prednisolone Antibiotics
...ds ...taph. ...ococci	Prednisolone Antibiotics
	Prednisolone Triamcinolone ACTH Antibiotics
	Prednisolone, Azathioprine Cyclophosphamide 6 Mercaptopurine Antibiotics, γ-globulin 250 mg every 6 weeks
...ia	Antibiotics, Frusemide

Patients 7 and 9 had bilateral inguinal herniae The onset of generalised oedema followed a Ramstedt's operation for pyloric stenosis in Cases 4 and 8.

The cause of death was septicaemia in at least 6 cases (Table 2). Peritonitis occurred in 5 cases. A list of the causative organisms is given. In 5 cases the infants were said to have died suddenly A number of infants (Table 2) had unexplained diarrhoea and vomit ing with dehydration and sometimes marked hyponatraemia. They often lost considerable weight during these periods with little change in the state of their oedema.

There was no history of Fenno-Scandinavian ancestry in any family Patients 1 and 2, and 7 and 8 were sibs There was no consanguinity in either case Patient 3 came from a remote village in North Wales where there was much inter-marriage and although not proven the parents admitted the possibility of consanguinity A second infant was admitted to a local hospital at 2 months of age with a urinary infection and a "heavy cloud" of albumen in the urine was noticed. She died suddenly at home a few weeks later with gastroenteritis. No oedema or ascites was mentioned. A third child is normal

Patient 8 survived till 5 years of age and died of renal failure. His eventual height at 5 years was 34 inches which is well below the third percentile

Laboratory Data

Table 3 demonstrates the urinary findings. R.B.C.s and/ or W.B.C.s were a prominent feature also casts were present in 5 out of 9 cases. A urinary infection was documented in 5 cases. Reducing substances were not found. Two cases had an increase in amino-acid excretion. The amount of protein varied during the course of the illness, up to 4 g per day were excreted.

The serum proteins showed a nephrotic pattern with low albumen and gamma globulin and raised alpha 2 globulin. Serum IgM measured in Case 9 was markedly raised at 140 mg/100 ml with IgG 120 mg/100 ml and IgA 52 mg/100 ml. The blood urea varied with the state



Fig 1 Case 3 Mesangial sclerosis crowding of lobules obliteration of capillary loops

Fig 2 Case 4 Two populations of glomeruli (see text) Ectasia of tubules

Fig 3 Case 8 Advanced glomerulosclerosis.

Fig 4 Case 9 Hypertrophic interlobular artery

of hydration Patient 8 died of progressive renal failure with a terminal blood urea of 470 mg/100 ml Case 5 had a blood urea terminally of 220 mg/100 ml It had been normal $3^1/_2$ weeks earlier and presumably was related to dehydration Severe hyponatraemia occurred in 4 cases and will be discussed later Serum sodium was not measured in ? cases The Wasserman reaction was negative in the 7 cases in which it was done Serum cholesterol typically was relatively low initially rising as the disease progressed. Case 9 being the most recent was the most fully investigated Serum complement BIC/BIA was 60 mg/100 ml (normal for his age (20)) The index of selectivity of proteinuria was 0 06 (normal) ^{131}I hippuran renogram showed a prolonged collection and a blood clearance of ?/3 normal Serum creatinine was 0 4 mg/100 ml

The I V P showed enlarged kidneys in ? cases (7 and 9) and a suggestion of a cystic process was seen in Case 7 and in Case 8 spidery calyces were commented on

Histological Studies

In 7 cases the kidneys were enlarged. In Case 1 the kidneys were normal size and in Case 8 who died of renal failure they were small

Renal histology

Case 1 (age 4 weeks) There was an increased cellular population of the glomeruli mainly due to mesangial cell proliferation Some ectasia of the tubules near the cortico-medullary junction was seen

Case 2 (age $7\,^1/_2$ months). More proliferation of the mesangial cells than the previous sibling. Again only patchy ectasia of tubules in the cortico-medullary junction

Case 3 (age 12 weeks) Marked mesangial sclerosis with crowding of the glomerular lobules. The capillary loops were bloodless The glomerular spaces were distended There was marked ectasia of the tubules which were lined by hyperplastic epithelium (Fig 1).

Case 4 (age 16 weeks) Two distinct populations of glomeruli (i) microglomeruli small immature tufts covered by cuboidal epithelium set in a distended capsular space (ii) the tufts filled the capsular space with many lobules and some mesangial cell proliferation. There was generalised ectasia of proximal tubules (Fig 2)

Case 5 (age 10 months) There was widespread glomerulitis characterised by varying degrees of increased cellularity Some distention of Bowman's capsule was seen with patchy ectasia of proximal tubules and widespread interstitial oedema and fibrosis.

Case 6 (age 8 weeks). Mild proliferation with some distension of Bowman's capsule was seen. There was marked ectasia of the proximal tubules.

Microdissection demonstrated a number of cystic dilatations in the proximal tubules and also some areas of what is considered typical of "microcystic disease".

Case 7 (age 10 months). Essentially similar to case 4 with 2 distinct populations of glomeruli. There was only slight tubular ectasia.

Case 8 (age 5 years). Advanced glomerulosclerosis. In those glomeruli which had not undergone complete hyaline sclerosis there were epithelial crescents. No tubular ectasia (Fig. 3).

Case 9 open biopsy (age 4 weeks). A large proportion of the glomeruli were small and immature with dilated capsular spaces—microglomeruli. Many were undergoing sclerotic changes or were completely hyalinised. The tubules were normal.

Autopsy (age 12 weeks). Patchy ectasia of the proximal tubules was now seen. Some glomeruli which were not hyalinised showed increased lobulation. Many lobular arteries showed a swelling of the intima which had formed a honeycomb pattern stretching across the lumen (Fig. 4).

Microdissection was also performed on Cases 1, 2 and 3 but the results were inconclusive.

Immunofluorescent studies

Case 7. No positive staining with anti-human conjugated antisera against gamma globulin or complement.

Case 9. Also negative for the presence of gamma globulin.

Treatment

All the patients died. Two cases (1 and 7) did not receive treatment (Table 2). Except in Case 8 antibiotics did not prevent septicaemia. Corticosteroids or immunosuppressive agents did not alter the course of the disease. Neither were diuretics very effective.

Case 8 was unusual in that despite early onset and persistent proteinuria, serious infections seemed to have been prevented by gamma globulin injections, 250 mg, weekly and antibiotics given for infections. He received prednisolone, unisone, azathioprine, cyclophosphamide and 6 mercaptopurine—none of which prevented the progression of the disease. Interestingly he developed chickenpox at 10 months of age while on unisone and cyclophosphamide with no serious effect.

DISCUSSION

The cases of nephrotic syndrome in infancy as described outside Finland do not have the same uniformity. It is difficult to find labelling factors which clearly differentiate them. The most obvious reason for wanting to classify them as to their similarity to the Finnish cases is for the purpose of genetic counselling. There is a familial tendency in the non-Finnish cases (12, 20, 29, 44, 45) but to a lesser degree than the incidence of recessive inheritance in Finland.

If a large placenta, low birth weight, early onset (i.e. before 3 months of age), an early death (before 4 years) and histological changes of mesangial cell proliferation, microglomeruli and ectasia of renal tubules in varying degrees of manifestation are taken as features typical of the Finnish cases, Cases 1, 2, 4, 6 and 9 might be the most similar. In Case 5, although the histology showed some distention of Bowman's capsule and slight tubular dilation, the relatively large birth weight and late onset (9 months) made her the least similar. Onset of the nephrotic syndrome at this age is even less common than early in infancy (31, 45). Case 3 (Fig. 1) in the light of recent identification of some infants with the nephrotic syndrome and diffuse mesangial sclerosis (13, 19) and focal glomerulosclerosis (13, 31) might also be treated as a separate group. Patients 7 and 8, who were siblings, were both well grown at birth. Despite early onset, patient 8 lived to die of uraemia and advanced glomerulosclerosis (Fig. 3) at 5 years. Presumably if a biopsy had been taken earlier in life it may have shown the microglomeruli and mesangial cell proliferation of his sibling. In the present small series four out of the nine occurred in siblings—2 siblings (cases 1 and 2) in the group of 5 patients similar to the Finnish infants and in the remaining 4 patients Cases 7 and 8, and as mentioned possibly Case 3, were familial.

Ectasia of the tubules is an interesting histological finding confirmed by microdissection studies (6, 8, 35, 36) and gave rise to the term "microcystic renal disease" (35). It is not seen in all of the Finnish cases (39, 19) and to much lesser extent in cases outside Finland (13, 31).

Similar ectasia can be seen in oligomega nephrony (40) which presents in infancy with uraemia and failure of renal acidification and in chronic nephritis (9 33 34) where ectatic tubules are hyperactive and compensating for the atrophic ones In our series significant ectasia was only seen in 3 cases and only slight in 5 others Present knowledge suggests that it is a secondary perhaps compensatory mechanism

Microglomeruli (Fig 2) where small immature tufts are perched at one pole of the distended capsular space (Cases 4 7 9) have a similar appearance to the glomeruli seen in congenital dysplasia (4) suggesting the reaction of glomeruli to an insult early in life Sometimes Bowman s capsule is distended but the glomeruli are of normal dimensions (Cases 3 5 6)

The marked thickening of the vessel walls in Case 9 (Fig. 4) seemed similar to the 2 siblings described by Hansen et al (20) They may represent secondary hypertensive changes It was noted that Case 9 had a raised blood pressure

In the CN earlier work of immunofluorescent studies by Kouvalainen (26) and Lange et al (27 28) gave positive results whereas most recent studies have not confirmed these (21 39 19) In our 2 cases the results were negative The fact that Hoyer et al (22) found evidence of immunological activity late in disease which was not prominent on earlier biopsies suggests that it is a secondary not causative phenomenon

The poor prognosis is a feature of the disease In the present series all have died Death was invariable in the Finnish cases the oldest survivor being 3 years and 10 months (18) At the time of reporting Worthen et al (47) had 3 out of 12 patients alive and Habib et al (13) 2 out of 14 In White s survey (45) 4 out of 17 non Finnish patients were alive all with active disease Fontaine et al (10) and Fournier et al (11) each described a single case with onset at 3 5 months who appeared to have a complete remission at 2 5 and 6 year follo up respectively

The cause of death is most commo infection which also has serious effects older nephrotics (46) Low IgM compleme and deficient phagocytosis seem importa reasons for the newborn s proneness to i fection (Davies (5)). It is difficult to kn how long these adverse factors continue operate in the older infant Typically in t CN IgM is elevated and IgG is low (3 The raised IgM seems to confer no parti ular advantage and may well be a respo to infection (15) The low IgG levels cou well be the most important factor Case was the only patient not to die of infecti and survived long enough to die of chron renal failure He received regular injectio of gamma-globulin till his death This mig be a worthwhile form of supportive therap combined with diuretics and appropriat antibiotics

Also sudden death for no appare reason may occur This may be due to a infection such as peritonitis the severity which is underestimated and/or the electro lyte changes (14) Hyponatraemia is parti cularly striking Diarrhoea and vomiting, prominent occurrence could be related t oedema of the bowel wall or as a manifesta tion of a systemic illness Indeed oedema o the bowel wall may have been the precipi tating factor in genetically predisposed indi viduals in the 2 cases who had pylori stenosis (4 and 8) Moncrieff et al (31) also had 2 cases with CN and pylori stenosis In Table 2 the incidence of tubular ectasia hyponatraemia diuretic therapy and diarrhoea and/or vomiting can be compared With the small numbers no definite correla tion between tubular ectasia and hyponatraemia can be made and fluid loss from diarrhoea and vomiting seems a more likely cause of hyponatraemia From the same Table septicaemia seems the most likely cause of the sudden death Generally full strength plasma is contra-indicated in the

nephrotic syndrome because of its sodium content but it may be indicated as a resuscitative procedure in a desperately ill infant who has hyponatraemia hypovolaemia and hypoalbuminaemia

Corticosteroids and immunosuppressives had no effect on the course of disease and could have been harmful. In the nephrosis of infancy it is unknown how long the insult has been active before birth probably long enough for the damage to have become irreversible Recently there has been a report of successful renal transplantation in 3 children (22) which may offer some hope

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A RECESSIVE DISORDER WITH GROWTH AND MENTAL RETARDATION PECULIAR FACIES ABNORMAL PIGMENTATION HEPATIC CIRRHOSIS AND AMINOACIDURIA

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ABSTRACT Tay C. H., Rajagopalan K. McEvoy-Bowe E. Tock, E. P C. and Da Costa, J. L. (Departments of Medicine Biochemistry and Pathology University of Singapore Singapore, and the Skin Clinic, General Hospital, Johore Bahru, West Malaysia). A recessive disorder with growth and mental retardation peculiar facies, abnormal pigmentation, hepatic cirrhosis and aminoaciduria. Acta Paediatr Scand, 63: 777 1974.—Two Indian teenage sisters from West Malaysia were recently found to have a previously undescribed syndrome consisting of (1) growth retardation and hypogonadism, (2) mental deficiency (3) peculiar facies consisting of microcephaly triangular shaped face prominent eyes, hypoplastic alae nasi, small 'pinched' nose tiny mouth and large pegged-shaped incisors, (4) abnormal pigmentations such as café-au-lait spots, multiple lentigines, peripheral vitiligo and premature canities, (5) abnormal limbs consisting of trident hands, hypoplastic transverse palmar creases, large big toes and short, stubbed small toes, (6) liver involvement with fatty infiltration and hepatic cirrhosis with hypersplenism, (7) raised serum globulins and serum immunoglobulins, and (8) hyperaminoaciduria mainly of taurine, β-aminoisobutyric acid and glycine. This syndrome is probably due to a recessive autosomal trait.

The relationship of this to other similar conditions, especially the Bird-Headed Dwarfism is briefly discussed.

KEY WORDS: Hereditary abnormalities, growth and mental retardation, abnormal facies, abnormal pigmentation, aminoaciduria

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A RECESSIVE DISORDER WITH GROWTH AND MENTAL RETARDATION PECULIAR FACIES ABNORMAL PIGMENTATION HEPATIC CIRRHOSIS AND AMINOACIDURIA

C. H. TAY K. RAJAGOPALAN E. McEVOY-BOWE, E. P. C. TOCK, and J. L. Da COSTA

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A previously undescribed syndrome characterized by an autosomal recessive trait, mental and growth retardation, peculiar facies, abnormal limbs, pigmentary disorder, hepatic cirrhosis, hyperglobulinaemia and hyperaminoaciduria was recently found in two Indian sisters from Johore Bahru, West Malaysia. The essential features of this syndrome as well as the differential diagnosis are presented and discussed.

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Case I (fig. 1 arrowed)

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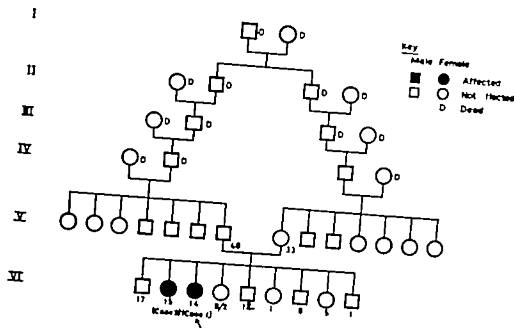


Fig 1 The family pedigre

of a triangular-shaped face prominent eyes hypoplastic alae nasi small pinched nose tiny mouth and large pegged-shaped incisors (Fig 2) Her body was slender She had trident hands hypoplastic transverse palmar creases (Fig. 3) Large big toes and short stubbed small toes (Fig. 4)

Dermatological findings

Large number of cafe-au-lait pigmentations each measuring 2 to 8 cm in diameter and multiple pinpoint lentigines both found at birth were observed on the trunk and the extensor aspects of the arms and legs Five years ago hairs of her scalp eyebrows and lashes and the limbs turned grey prematurely (canities) and at the same time vitiliginous lesions appeared on her shins knees extensor surfaces of arms and the upper chest wall (Fig 4) Biopsies of these lesions were consistent with vitiligo and lentigines.

Other findings

Moderate anaemia a left conductive deafness a firm liver of 2 cm below the right costal margin and an enlarged spleen of 10 cm were found. All the other systems were normal.

Laboratory findings

Haemoglobin 9 6 g/100 ml Total white cell count 2 900/mm^3 platelets 45 000/mm^3 Differential white count reticulocyte count and Hess test were normal Results of peripheral blood film and bone marrow examination were consistent with an iron deficiency anaemia Serum iron was 45 μg/ml. Occult bleeding was not detected in the stools. Liver function tests. Serum bilirubin 0 7 mg/100 ml SGOT 298 units/ml SGPT 194 units/ml Serum alkaline phosphatase 28 K A U Serum albumin 3 2 g/100 ml Serum globulin 4 1 g/100 ml (serum gamma-globulin 2.3 g/100 ml Serum immunoglobulins were raised—IgG 4 800 mg/100 ml IgA 528 mg/100 ml and IgM 100 mg/100 ml (normal range for her age and sex—IgG 680 to 1 152 mg/100 ml IgA 137 to 387 mg/100 ml and IgM 56 to 98 mg/100 ml) Barium study revealed oesophageal varices but no other lesions were detected. Liver biopsy showed a multinodular hepatic cirrhosis without

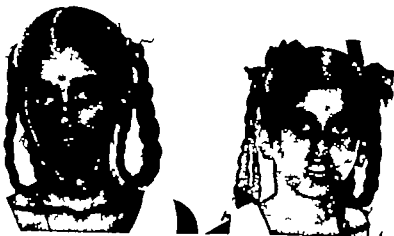


Fig 2 The peculiar facies of Cases 2 and 1 Note the small head triangular face prominent eyes small pinched" nose tiny mouth large peg-shaped incisors and premature greying of the hairs of the scalp and eyebrows.



Fig 3 The trident hands with hypoplastic transverse palmar creases of both patients.

...y acute necrosis, biliary stasis or abnormalities of the ...terlobular ducts. Histochemical study failed to detect ...y infiltrative disorders. Renal function was normal. ...er bone age was estimated to be 11 years (7). Amino-...id assay of her blood and skin tissues revealed ...ormal content but a urinary chromatogram (9) showed ...at she excreted excessive amounts of taurine and ...ycine as compared with her normal sister—1.60 ...micromole per μg creatinine of taurine (normal 0–1.20 ...moles/μg creatinine) and 2.60 μmoles/μ creatinine ...f glycine (normal 0.15–2.50 μmoles/μg creatinine) (Fig 5). Levels of urinary ethanolamine, histidine, ...ysine and other basic amino acids were also elevated. There was no glycosuria or phosphaturia. Other investigations including haematological, biochemical, metabolic, immunological, radiological and chromosomal studies were essentially normal.

Case 2 (Fig 1)

...ler sister of patient 1 and 2nd in this family, this ...-year-old Indian girl was found to have the same ...ase.

...velopmental retardation

...wth retardation. Born prematurely at 8 months, she ...d weighed 1.14 kg. Her developmental milestones ...re similarly delayed and her somatic growth was ...ed. On examination she weighed 25 kg and ...asured 135 cm in height (both below the 3rd percentile). Her breasts were small and undeveloped and there ...ere no axillary or pubic hairs. Menstruation had not ...menced.

...ental retardation. Her intelligence was subnormal. ...er I.Q. was 70.

...normal facies and limbs

The facial appearance and limb abnormalities were identical with those of Case 1, the skull circumference ...asured 47.5 cm (Figs 2, 3 and 4).

Dermatological finding

...imilar cutaneous lesions (Café-au-lait spots, lentigines ...d vitiligo) and premature canities were also found, ...ough fewer and less extensive (Figs 2 and 4).



Fig 4 Multiple lentigines and vitiligo patches on the lower legs, extensor aspect of arms and upper chest wall in Case 1 (right). Case 2 (left) has similar bu... less extensive skin lesions on the chest and legs. Not... the abnormally large big toe and stubbed small toes more evident in Case 2.

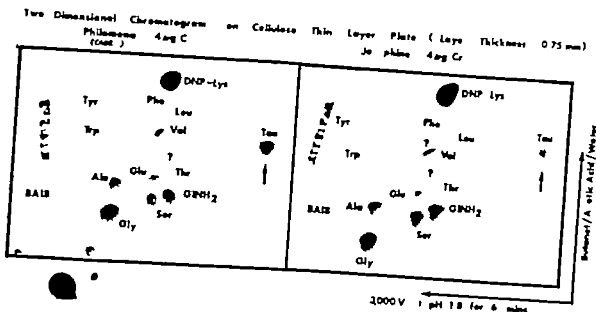


Fig 5 Two-dimensional urinary chromatogram of Case 1 (left) and a normal sister (Josephine) (right) Note Case 1 has denser taurine (Tau) (arrowed) and glycine (*Gly*) spots than her sister

Other findings

Except for a hepatomegaly of cm no other physical abnormalities were detected. She was not anaemic or jaundiced.

Laboratory findings

All the laboratory investigations done in the previous case were also carried out in this patient The following results were abnormal. SGOT 194 units/ml SGPT 145 units/ml serum alkaline phosphatase 21 K A U serum albumin 3 3 g/100 ml serum globulin 3 8 g/100 ml (serum-gamma-globulin 1 9 g/100 ml) Serum IgG 7360 mg/100 ml serum IgA 680 mg/100 ml serum IgM 114 mg/100 ml Liver biopsy revealed liver cells with vacuolated cytoplasm due to fatty infiltration Her bone age was 12 years (7) Renal functions were normal As in case 1 the blood and tissue amino acid content was normal but urinary chromatogram study revealed the presence of 2 08 nanomoles per µg creatinine of taurine (normal range 0–1 20 nmoles/µg creatinine) 2.75 nanomoles per µg creatinine of β-aminoisobutyric acid (normal range 0 05–0 70 nmoles/µg creatinine) and excessive amount of basic amino-acids

Family study

As shown in Fig. 1 Cases 1 and were 2 of the 9 siblings in this family Both parents apparently normal were related 4 generations ago Clinical and laboratory examinations of the parents and the rest of the living sibs were essentially normal. However the mother and one sister (Josephine) were found to excrete top normal amount of glycine in their urine—2.50 nanomoles per µg creatinine Both had no evidence of liver disease

DISCUSSION

The main features in the two sisters were (1) moderate mental deficiency (2) growth retardation and hypogonadism (3) peculiar facies consisting of microcephaly triangular shaped face prominent eyes hypoplastic alae nasi small pinched nose tiny mouth and large pegged shaped incisors (4) abnormal limbs showing trident hands hypoplastic transverse palmar creases large big toes and short stubbed small toes (5) abnormal pigmentations—café-au lait spots multiple lentigines peripheral vitiligo and premature canities (6) the hepatic lesions—fatty infiltration and liver cirrhosis (7) hyperglobulinaemia and hyperimmunoglobulinaemia and (8) hyperaminoaciduria mainly of taurine β-aminoisobutyric acid and glycine In addition Case 1 had pancytopenia hypersplenism portal hypertension and a left conductive deafness The syndrome is probably due to a recessive autosomal trait

The combination of all these features in a single individual has to our knowledge not been previously documented There are

Table 1 *Differential diagnosis*

Diseases ( )=Reference	Autosomal recessive inheritance	Growth retardation	Mental defects	Abnormal facies	Other features
Present disease	+	+	+	+ (microcephaly prominent eyes small "pinched" nose triangular face tiny mouth & large pegged incisors)	Hypogonadism, trident hands abnormal toes, café-au lait spots, lentigines vitiligo caroties, hepatic cirrhosis pancytopenia hypersplenism, hyperglobulinaemia, hyperimmunoglobinaemia aminoaciduria
Bird headed dwarfism (4, 10, 14)	+	+	+	+ ("Bird-head" profile)	Trident hands, skeletal defects, hypodontia brownish pigmentations with white macules, caroties, pancytopenia with hypersplenism, premature senility
Anhidrotic ectodermal dysplasia (15)	+	+	+	+	Sparse hairs hypohidrosis anodontia
Berlin s syndrome (1)	+	+	+	+	Mottled skin hypohidrosis thickened palms and soles
Rothmund Thomson syndrome (12)	+	+	+	0	Cataract, keratosis, telangiectasia, light sensitivity pigmentations
Bloom syndrome (11)	+	0	0	0	Erythema, light sensitivity telangiectasia
Cockayne s syndrome (2)	?	+	+	+ ("Mickey Mouse facies")	Light sensitivity Skeletal defects
Cornelia de Lange syndrome (8)	+	+	+	+ (Typical facies)	"Marbling" of skin Skeletal defects hypertrichosis
Focal dermal hypoplasia (6)	+	+	+	+ (Facial asymmetry)	Skeletal abnormalities ocular defects dystrophy and atrophy of skin, hair and nails
Treacher Collins syndrome (3)	0 (Autosomal dominant)	0		+ ("Bird-like")	Skeletal defects Abnormal hair pattern
Hallermann Streiff syndrome (5)		+	?	+ ("Parrot" facies)	Alopecia, cataract, small eyes, absent teeth

however a number of rare conditions which show close resemblance to the present disease with regard to the presence of an autosomal recessive inheritance growth retardation mental defects abnormal facies and other physical abnormalities As shown in Table 1 they are the 'Bird-headed dwarfism (4 10 14) the Anhidrotic ectodermal dysphasia (15) the Berlin s syndrome (1) Rothmund Thomson syndrome (12) Bloom s syndrome (11) Cockayne s syndrome (2) Cornelia de Lange syndrome (8) Focal dermal hypoplasia (6) Treacher-Collins syndrome (3) and the Hallerman-Strieff syndrome (5) These conditions differ from ours because they have a distinctive facial appearance with systemic involvement not detected in our cases e g involvement of

nails hair shafts teeth eyes sweat glands skeleton nervous system etc

It is interesting to note that Bird-headed dwarfism has many features with striking similarity to the present disease and hence our patients may be a variant of the former condition However Bird headed dwarfs have characteristic features consisting of a central beak like protrusion of the face prominent nose bilateral ptosis receding chin and low set deformed ears Amino-aciduria hyperglobulinaemia and hypogonadism are absent but most patients have hypodontia and various skeletal defects

In our patients the presence of café au lait pigmentations multiple lentigines peripheral vitiligo and premature canities do not seem to be related to any known diseases with which they are commonly associated The cause of the liver disease too is obscure since all known conditions had been excluded

The presence of pancytopenia iron deficiency anaemia and the abnormal liver function tests (including the elevated levels of serum globulins and serum immunoglobulins) in Case I are most certainly due to the hepatic cirrhosis and hypersplenism Case 2 with the milder form of the hepatocellular disease showed less functional disturbance

In the absence of hyperaminoacidaemia, the high urinary output of taurine β-aminoisobutyric acid glycine and other basic aminoacids in these girls may be a reflection of their liver disease and tissue catabolism (13) On the other hand the abnormal aminoaciduria may be an integral part of the syndrome since substantial glycinuria was detected in the mother and an unaffected sister who both lacked signs of liver disease

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CASE REPORT

INJURY PATTERN FOLLOWING PAROXYSMAL SUPRAVENTRICULAR TACHYCARDIA IN A CHILD

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From the Alder Hey Children's Hospital, Liverpool, England

ABSTRACT Ghosh, A. and McCandless, A. E. (Alder Hey Children's Hospital, Liverpool, England). "Injury Pattern" following paroxysmal supraventricular tachycardia in a child. Acta Paediatr Scand, 63: 783, 1974.—Electrocardiograms recorded after the control of a prolonged attack of paroxysmal supraventricular tachycardia lasting for five days in a child showed T wave inversion, notching and prolonged duration of P wave and an "injury pattern". These electrocardiographic changes described mostly in adults are uncommon in children. Antibody titre of mumps rose from 1/40 after admission to 1/320 thirteen days later suggesting recent mumps infection. Mumps myocarditis may have caused paroxysmal supraventricular tachycardia in this child

KEY WORDS: Paroxysmal supraventricular tachycardia, mumps

Development of an "injury pattern" in electrocardiogram after an attack of paroxysmal supraventricular tachycardia has been described mostly in adults. Paroxysmal supraventricular tachycardia has only rarely been attributed to mumps. We describe both these associations in a girl, seven years of age, weighing 22 kg.

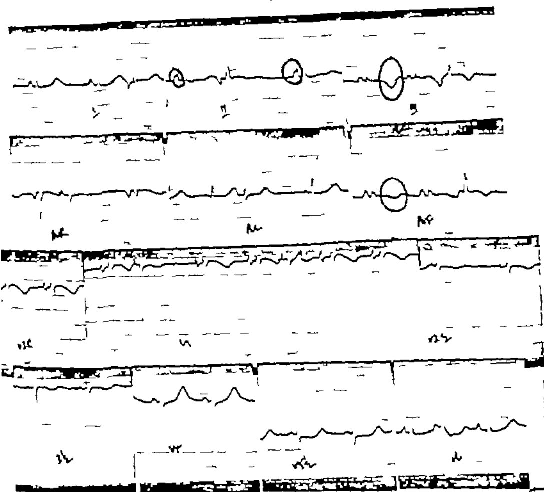
CASE REPORT

E. L. was admitted to hospital with a history of having been unwell at home for five days with an upper respiratory tract infection, vomiting and thumping in her chest. On admission she had enlarged and inflamed tonsils. Her pulse rate was 260 per minute. The liver edge was 7 cm below the right costal margin and was tender. Heart borders were within normal limits by X-ray and no cardiac murmur was heard. During the previous three months the child had had five or six attacks in which she had complained of a sudden onset of thumping in her chest. She had then slept for about an hour and on awakening had felt well. The clinical impression on admission was of several previous attacks of paroxysmal tachycardia and a current attack of 5 days duration which had led to congestive heart failure. Electrocardiography (Fig. 1) confirmed the clinical diagnosis of paroxysmal supraventricular tachycardia and she was digitalised. She was given 0.05 mg of digoxin on admission, 0.025 mg 6 hours and 12 hours later and for subsequent 5 days 0.01 mg twice a day.

Within 6 hours her pulse rate fell to normal and by the next morning she was symptomless and the liver was no longer enlarged or tender. Subsequent electrocardiograms showed T wave inversion, notching and prolonged duration of P wave and "injury pattern". Viral studies revealed rise in the antibody titre of mumps from 1/40 after admission to 1/320 thirteen days later.

DISCUSSION

The post-paroxysmal electrocardiographic changes of T wave inversion on the second (Fig. 2) and the eighth (Fig. 3) days suggest myocardial strain caused by prolonged paroxysmal tachycardia at 260 beats a minute for five days. This change is prominent



ig. 2. 2nd day. In Lead II, notched and wide P waves. In Lead III and VF inverted T waves.

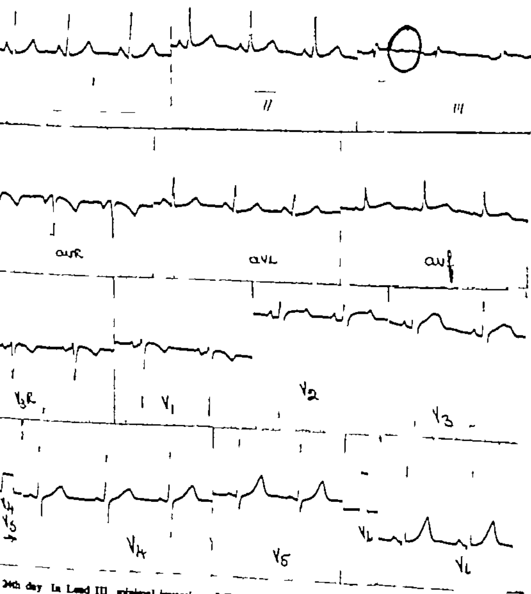
resent in the later electrocardiogram (Fig.).

The possibility of electrocardiographic changes due to digoxin was considered but in digitalis effects P wave changes and injury pattern are not seen and T wave appearances are unlike those present in these electrocardiograms. Moreover this child had a very small dose of digoxin.

The Viral Studies in this child indicate recent mumps infection. Rosenberg (5) and later Bengtsson & Orndahl (1) found electrocardiographic changes in several cases of mumps which suggested myocardial involvement and Roberts & Fox (4) reported a case of mumps in which autopsy showed diffuse interstitial myocardial fibrosis. Mumps myocarditis may therefore have caused the last episode of paroxysmal tachycardia in this child, the cause of the previous paroxysms remaining unknown.

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24th day. In Lead III minimal inversion of T

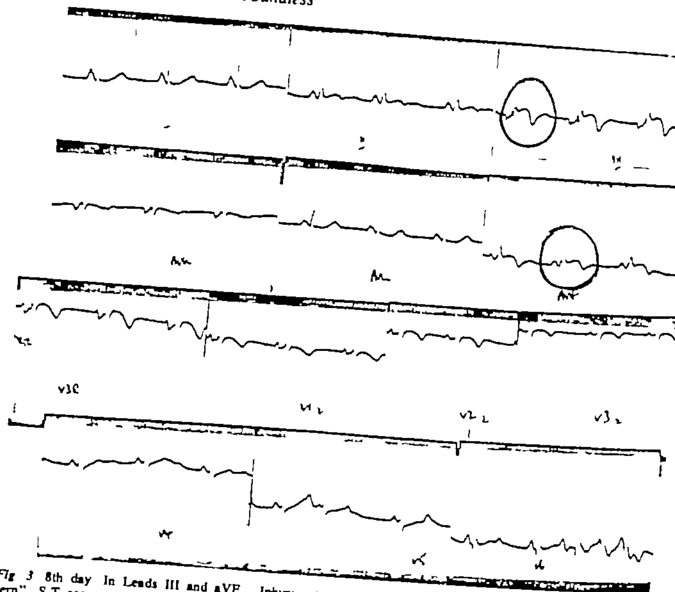


Fig 3 8th day In Leads III and aVF "Injury pattern" S-T segments are raised and T waves are inverted (note that P waves in Lead II are normal)

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ig. 1. Erythema annulare centrifugum. Appearance at first admission, Feb. 21 1972. (*a*) This was the first lesion here 2 weeks old. (*b*) One lesion a few days old.

eks these increased in size and new ones appeared elf on the face and on the trunk. On her first admission to hospital on Feb. 21 the child's general ondition was normal. The largest annular lesion on the ce was 4 cm in diameter and had an elevated red margin and central clearing, fine scaling and moderate pigmentation (Fig. 1). There were several smaller annular lesions on the face and trunk. A week later several new lesions appeared, others increased in size and coalesced with adjacent ones. The general condition was still unaffected and the girl increased in weight on schedule. She was breast fed only during her first week of life. Except for water-soluble A and D vitamins no drugs had been given either to the child or to the mother.



Fig. 2. Erythema annulare centrifugum. Histological examination revealed dense cellular infiltrate in the dermis especially around the vessels. The epidermis displayed oedema without liquefaction degeneration of the basal layer.

Physical examination revealed nothing abnormal except for the skin lesions on the face, scalp, neck and trunk (Fig. 1). Chest X-ray normal.

Laboratory investigations. Blood: Haemoglobin 16.3 g/100 ml, white blood cell count 10300 (neutrophils 16.5%, eosinophils 4%, basophils 0.5%, lymphocytes 77%, monocytes 1.5% and plasma cells 0.5%). Erythrocyte sedimentation rate 3 mm/hour. Urinalysis gave normal results, culture negative. Stool examinations showed no ova, viruses or parasites. Bacterial cultures from the skin and mouth were negative. The Sabin-Feldman toxoplasma dye test and serologic tests for syphilis gave negative results. Scrapings from ringed lesions were repeatedly negative for fungi. During a short period in early March scrapings contained yeast-like organisms which did not grow in cultures, however.

Histological examination. A biopsy from a typical lesion on the back taken Feb. 21 showed an oedematous epidermis with normal stratification (Fig. 2). A dense cellular infiltrate predominantly consisting of lymphocytes, histiocytes and reticulum cells was found subepidermally throughout the entire part of the dermis submitted. It was more pronounced around the dermal vessels, which showed signs of leucocytoclastic vasculitis.

Virologic studies. Complement fixation tests for the following viruses gave low titres: adeno- and cyto

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CASE REPORT

ERYTHEMA ANNULARE CENTRIFUGUM COINCIDENT WITH EPSTEIN BARR VIRUS INFECTION IN AN INFANT

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ABSTRACT Hammar H (Department of Dermatology Karolinska Sjukhuset, Stockholm, Sweden) Erythema annulare centrifugum coincident with Epstein-Barr virus infection in an infant. Acta Paediatr Scand 63 788 1974.—Persistent annular erythemas include various more or less distinctly defined clinical entities mostly found in adults but are sometimes seen in infants. Erythema annulare centrifugum is distinguished as one group among these dermatoses. The pathogenesis of the annular erythemas is not clearly understood A two-week-old girl who developed recurrent erythema annulare centrifugum during her first 6 months of life is described. Significant changes in the titres of anti-Epstein-Barr virus antibodies were coincident with the clinical course The general health of the girl was not affected and there were no signs of infectious mononucleosis either in the girl or in her mother The eruption left an apparently normal skin except for one lesion with atrophy and telangiectases one year after its first appearance The coincidence of the significant rise and fall of anti-Epstein-Barr antibody titres with the clinical appearance of erythema annulare centrifugum strongly suggests a pathogenetic effect of the virus on the skin eruption.

KEY WORDS Erythema annulare centrifugum persistent annular erythema Epstein-Barr virus, serology infancy

Persistent annular erythemas include a large number of eponyms of more or less distinctly defined clinical entities These are mostly found in adults but are sometimes also seen in infants (2 5a) Shelley (20) divided the different variants into three groups erythema annulare centrifugum (4) erythema gyratum repens (7) and erythema chronicum migrans (13) which should be distinguished from similar eruptions including erythema rheumaticum marginatum erythema multiforme lupus erythematosus cutaneous lymphoma sarcoidosis and tinea Others have delineated familial annular erythema as a distinct group (2 8) The pathogenesis of the annular erythemas is not clearly understood as pointed out by White & Perry (22) This is especially true for the clinical variants designated erythema annulare centrifugum There is a v documented relationship between erythe gyratum repens and internal carcinoma 17) and an association has also been fo between erythema chronicum migrans an preceding tick-bite (9) with possible inocu tion of a rickettsia species (5 18)

A new aetiological factor in erythe annulare centrifugum is presented here T report concerns an infant with signs Epstein Barr virus (EBV) infection associat in time with the skin lesions

CASE REPORT

The patient, a girl born Jan 1 197? after a norm pregnancy and delivery developed three small slight elevated erythematous patches in the left tempora region at the age of weeks During the following

ther had this disease but neither the other nor the child had any general symptoms and furthermore the test for heterophile antibodies (monospot) was negative. Infectious mononucleosis is rare in infants and in children under 5 years of age the serological findings are negative in otherwise probable cases (3, 21). The haematological data in the present case showed a preponderance of mononuclear blood cells. This is the rule, however, in the patient's age group. Abnormal mononuclear cells were not found.

The rash seen in infectious mononucleosis is a mostly evanescent macular of maculopapular exanthema, and sometimes morbilliform, urticarial or scarlatiniform eruptions are seen (11, 14). Our patient showed none of these alterations. The dermal infiltrate observed had a formal resemblance to smear pathology of lymph nodes in infectious mononucleosis (6). From this dicussion there would seem to be only circumstantial evidence of a diagnosis of infectious mononucleosis in our case.

The macroscopic and histological features of the eruption fit well with the morphological characteristics of erythema annulare centrifugum (12, 20). Lupus erythematosus and cutaneous lymphoma, which were discussed initially as differential diagnoses were excluded because of the absence of general signs and symptoms. Erythema multiforme was less probable from the histological findings of no epidermal changes in the basal layer and a comparatively slight vascular reaction in relation to the dense dermal infiltrate. A drug eruption was excluded by the negative history and the subsequent course. Infectious maculopapular exanthemas of viral or bacterial organ are mostly of short duration and they show only an unspecific histological picture (1, 12, 15, 16). Syphilis makes an exception, however, but this disease was excluded by the negative serology in mother and child.

The coincidence of the significant rise and fall of anti EBV antibody titres with the clinical appearance of erythema annulare centrifugum during a limited period strongly suggests a pathogenetic effect of the virus on the skin eruption.

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Fig 3 Erythema annulare centrifugum (*a*) Appearance on March 1 1972 (*b*) Appearance on March 14 1972

megaloviruses herpes simplex varicella and herpes zoster viruses and polio virus 3 Virus isolation was unsuccessful. These studies were also made in the child s mother with the same result

In the child anti EBV antibody titres were as followed March ? 1 40 March 15 1 ≥ 160 and June 16, 1 40 Titres in the mother were March 3 1 ≥ 160 and June 16 1 ≥ 160 A screening test for infectious mononucleosis monospot was negative on all these occasions. The child s father had been ill almost continuously with recurrent fevers and sore throats during the autumn of 1971 No diagnosis was made The mother did not have any signs of illness during her pregnancy The EBV titres indicated that both the mother and the patient had recently been infected with EBV

Subsequent course The culmination of the sl disease passed during the middle of March (Fig. On March 24 the initial lesion in the left temporal gion showed a blanched atrophic centre with m telangiectatic vessels surrounded by a cyanotic, sligh elevated margin Other lesions cleared without signs atrophy after one to several months duration Dur April and May some new lesions appeared. No lesio were seen after June 16 and all had disappeared Aug 22 except for the telangiectatic atrophic patch the left temporal region (Fig. 4) At a follow-up Feb 22 1973 the changes in this area were seen be less conspicuous The child was healthy showing no mal development and no sequelae in other parts of th skin



Fig 4 Erythema annulare centrifugum Appearance on Aug. 22 1972

COMMENTS

EBV infections in infants seem to be unusual Shapiro et al (19) examined anti EBV anti body titres in 8 mothers and their 9 childre during the children s first year of life A birth the titre approximated that of th mother The titres then decreased in the firs 4–5 months In the present case the titre changed in a different way however indicat ing that the patient had an EBV infection concomitant with the skin lesions

Henle et al (10) have attributed EBV an essential role in infectious mononucleosis The clinical data might infer that the patient s

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CASE REPORT

CONGENITAL CYSTIC ADENOMATOID MALFORMATION OF THE LUNG

A Report of Two Cases

HELGE MICHALSEN

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ABSTRACT Michalsen, H. (Department of Paediatrics, Ullevål Hospital, Oslo, Norway). Congenital cystic adenomatoid malformation of the lung. Acta Paediatr Scand, 63: 793, 1974.—The clinical picture of the congenital cystic adenomatoid malformation of the lung is reviewed. Two patients suffering from this entity are presented, the first patient was a newborn girl with acute respiratory distress shortly after birth, and the second patient was 12 months old and had a history of repeated pulmonary infections. The importance of, and difficulty in, differentiation between diaphragmatic hernia and the congenital cysts of this type in newborn infants is emphasized.

KEY WORDS: Congenital pulmonary cysts, neonatal respiratory distress, congenital cystic adenomatoid malformation of the lung

Congenital pulmonary cysts are rare and are by far outnumbered by the acquired cysts of the lung. The congenital cystic adenomatoid malformation of the lung (CCAM) is a separate entity among the various congenital forms of cystic disease of the lung. This condition was first described in 1949 (3) and since then there have been several reports dealing with this clinical and pathological entity (2, 4 5 7).

CCAM may affect any lobe there appears to be no predilection for any particular lobe Bilateral involvement of the lungs has been described only once (3) Both sexes are equally often affected The involved lobe is increased in size and weight, it is firm and rubbery of consistence and there are multiple cysts of varying size Solid types of adenomatoid malformation has recently been reported (7) The cysts are lined by a pseudostratified columnar epithelium which may form papillary projections into the cyst lumina, and there is no supporting cartilage to be seen (2, 4 5 7)

The patients may be stillborn or die immediately after birth In the living newborn a state of acute progressive respiratory distress is common Some patients have been recognized in whom the symptoms were absent in the neonatal period and the diagnosis established only after a period characterized by persistent or repeated pulmonary infections Fetal hydrops and anasarca have been described and hydramnios is often present, these findings being most often reported in the stillborn cases of CCAM (4 5)

The increasing neonatal respiratory distress is the most impressive clinical pattern of the CCAM. Owing to the lack of cartilaginous support, which permits collapse of the bronchi during expiration, air will be trapped in the cysts The cysts thus increase in size

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CASE REPORT

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A Report of Two Cases

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The increasing neonatal respiratory distress is the most impressive clinical pattern of the CCAM Owing to the lack of cartilaginous support, which permits collapse of the bronchi during expiration air will be trapped in the cysts. The cysts thus increase in size



Fig 1 X-ray of thorax from case I The left hemi thorax contains airfilled cavities and there is a shift of mediastinum towards the right side The right lung is partly atelectatic The upper 2/3 of the abdomen shows an apparently normal gas distribution

and the tension will eventually cause a shift of the mediastinum to the opposite side Herniation of the involved lung to the opposite side is frequently seen These changes cause tachypnoea and dyspnoea in the infants The symptoms may progress rapidly and make the condition of the infant critical

X-ray examinations are essential to the diagnosis The multiple cysts are visualized as a collection of translucent areas within the hemithorax the heart and mediastinum are displaced towards the opposite side and herniation of the involved lung may be seen These findings may mimic the radiographic findings in diaphragmatic hernia, and the distinction between these conditions is extremely important In doubtful cases radiographic studies with contrast media may afford the necessary evidence for the correct diagnosis

CASE REPORTS

Case 1

was a girl born on April 10th 1973 Pregnancy and delivery were uneventful Her weight was 3280 g and her length 51 cm She was given an Apgar score of 7 both after 1 and 5 minutes of life She was transferred to the paediatric department because of increasing cyanosis and respiratory distress There was a marked difference in the findings over the left and right hemithorax, the percussion note being dull and the respiratory sounds weak over the left lung X-rays of the thorax revealed airfilled cavities in the left hemithorax and the patient was considered to have a diaphragmatic hernia (Fig. 1) When she was 5 hours old laparotomy was performed with normal findings and therefore immediately followed by a thoracotomy The left thoracic cavity was filled by an emphysematous left upper lobe which was removed Microscopic examination revealed a multicystic lobe in which the cysts were lined by a cylindrical epithelium with small papillary projections into the lumina. No cartilage was seen (Prep no 5211 73 Pat-anat. Lab Ullevål Hospital) The postoperative convalescence was uneventful, and she was discharged 12-days-old Her development has been normal so far

Case 2

was a girl born on May 10th 1972 Except for her being extraordinarily thin she showed no signs of illness until the age of 11 months. From that time on she had repeated pulmonary infections. She was treated adequately with antibiotics with excellent effect but relapsed after few days without treatment She was admitted to our department at the age of 1? months She was pale and thin her length corresponded to the 75 percentile and her weight was 600 g below the ?.5 percentile A dull percussion note was found over the right lung On auscultation fine crepitations were disclosed and the respiratory sounds were weaker over the right lung. X-rays of the thorax revealed multiple translucent areas some of which contained fluid in the right lower lobe and there was a shift of the mediastinum towards the left side (Fig 2) She was treated with massive doses of antibiotics the possibility of staphylococcal pneumonia was dealt with through proper treatment. Her general condition improved and the signs of infections gradually subsided. Thoracotomy was performed 2 weeks after admission and the right lower lobe was removed. The resected lobe contained multiple cysts some of which were multiloculated Some of the cysts were filled with mucoid pus The cysts were lined by a cylindrical epithelium and cartilage was absent, but no papillary projections of epithelium was demonstrated (Prep no 6691 73 Pat-anat. Lab Ullevål Hospital). Respiratory syncytial virus was isolated from her expectorate but there were no signs of staphylococcal infection. The postoperative phase has been unev ful.



Fig 2. X-ray from patient 2. The lower part of the right hemithorax contains multiple cavities filled with air or fluid There is a shift of the mediastinum and trachea towards the left side

DISCUSSION

These patients represent two different patterns of the clinical presentation of CCAM The first patient had respiratory distress shortly after birth and her condition was critical before operation was performed. The clinical findings and the X-rays were misinterpreted as diaphragmatic hernia, and the correct diagnosis was established only after laparotomy Thus she underwent an unnecessary operation

The second patient had a history of repeated lung infections during the last month before admission, and was primarily thought to have pneumatocoeles caused by a staphylococcal pneumonia. The diagnosis of congenital cysts was established after microscopic examination of the removed lobe The examination failed to demonstrate the papillary projections of epithelium into the cyst lumina which are characteristic of CCAM but in every other respect the specimen was characteristic of this entity

These two problems of differential diagnosis are essential for the pediatrician It is important to determine whether the infant suffers from diaphragmatic hernia, as most surgeons are inclined to treat this condition through a laparotomy (2) The other diagnostic possibilities in the newborn include other types of congenital cysts congenital lobar emphysema and aplasia or hypoplasia of one lung (1 5 6) These conditions will usually provide characteristic radiographic findings and will be subject to thoracotomy as they are of pulmonary origin In doubtful cases X-rays of the abdomen are recommendable The presence of a normal gas distribution is a clue in favour of a pulmonary disease Studies with contrast media will provide conclusive evidence A small and scaphoid abdomen is seen in diaphragmatic hernia, and a normal appearance of the abdomen is a clue in favour of a pulmonary disease

There is apparently no disagreement in the literature concerning the treatment of CCAM Every patient has required surgical intervention with removal of the cysts the indication for operation being either the progressive respiratory distress in the newborn or the repeated infections in the older child. Total lobectomy of the involved lobe is recommended (4) The prognosis is good if the patient survives the first few hours of life

CONCLUSION

Congenital cystic adenomatoid malformation of the lung is a rare entity Some of the patients are stillborn. In the living infant there are two clinical patterns (I) acute and progressive respiratory distress in the newborn and (II) a course characterized by repeated pulmonary infections in the older children

X-ray examinations are essential for the diag nosis The treatment in the newborn is im mediate removal of the involved lobe while lobectomia in older children should follow a period of adequate treatment with anti biotics

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CASE REPORT

LSD INTOXICATION IN A TWO YEAR-OLD CHILD

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ABSTRACT Samuelsson, B. O (Department of Paediatrics, University Hospital, Uppsala, Sweden). LSD intoxication in a two-year-old child. Acta Paediatr Scand, 63: 797 1974.—*Report* on one case of LSD intoxication in a two-year-old child. The dose ingested was very high and the acute clinical picture of the patient seemed to be quite typical for a severe LSD intoxication. Despite the high dose, no long lasting or permanent sequelae seemed to have occurred.

KEY WORDS: LSD intoxication, children

There have been only a few reports of the effects of lysergic acid diethylamide (LSD) on children The symptoms and the complications are well documented for adults and seem to correspond widely to that in children

CASE HISTORY

This child, a boy was the first of twins, born 3 weeks pre term His birth weight was 2000 g and both delivery and neonatal period were uneventful.

Initial state and course

The boy had been completely healthy until his admission to the Department of Paediatrics at the University Hospital in Uppsala on July 23 1971 His age was 2 years and months, and he was admitted probably within 20 minutes after he had consumed 5 tablets each containing 0 4 mg LSD. A gastric lavage was immediately performed on arrival. No remains of the tablets could be identified.

On admission the boy was in a state of extreme excitability He also had mydriasis and pronounced ataxia He was unable to walk or grasp in a proper manner His respiration pulse heart rate and blood pressure were normal

About 10 minutes later i.e. about 1 hour after the intake of LSD, he had reached a state of total catatonia. No spontaneous movements were noted. His body and extremities rigidly remained in the position, in which they were placed At the same time the boy had become completely lost in himself without loosing consciousness. He had no contact with his surroundings. The mydriasis quickly became more pronounced, and his eyes were kept absolutely fixed. No reflexes could be elicited He responded only to strong tactile stimuli and strong pain. His face was peaceful and showed no signs of agony About 1 / hours after the intake of LSD his respiration became faster and irregular Tachycardia was also noted at this time, and his blood pressure had risen to 120 mmHg systolic.

Four hours after the consumption of the LSD tablets, spontaneous movements of the extremities and eyes returned, but there was a marked stiffness and resistance to passive movements of his limbs. The tendon reflexes, however were still absent. The respiration became normal 5 hours after LSD intake After 6 hours he fell asleep. During the following hours he slept peacefully except when the pulse and blood pressure were recorded. After 9 hours he had a 20-minute attack of repeated small convulsions in his extremities. Thereafter a continuous and steady normalization took place. The resistance to passive movements of the limbs however persisted for 16 hours and it was not until 48 hours after the LSD intake, that his behaviour general condition and neurological status had become completely normal.

Laboratory investigations during the acute phase

Except for a slightly low sodium concentration (131 mEq/l) the serum electrolytes were normal. The acid-base balance of the blood, all routine haematological and urinary analyses as well as serum creatinine and

X-ray examinations are essential for the diagnosis The treatment in the newborn is immediate removal of the involved lobe while lobectomia in older children should follow a period of adequate treatment with antibiotics

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CASE REPORT

LSD INTOXICATION IN A TWO-YEAR-OLD CHILD

B O SAMUELSSON

From the Department of Paediatrics University Hospital Uppsala S eden

ABSTRACT Samuelsson, B. O. (Department of Paediatrics, University Hospital, Uppsala, Sweden). LSD intoxication in a two-year-old child. Acta Paediatr Scand 63:797 1974.—Report on one case of LSD intoxication in a two-year-old child. The dose ingested was very high and the acute clinical picture of the patient seemed to be quite typical for a severe LSD intoxication. Despite the high dose no long lasting or permanent sequelae seemed to have occurred.

KEY WORDS: LSD intoxication, children

There have been only a few reports of the effects of lysergic acid diethylamide (LSD) on children The symptoms and the complications are well documented for adults and seem to correspond widely to that in children.

CASE HISTORY

This child a boy was the first of twins, born 3 weeks pre term. His birth weight was 2080 g and both delivery and neonatal period were uneventful

Initial state and course

The boy had been completely healthy until his admission to the *Department of Paediatrics at the University* Hospital in Uppsala, on July 23 1971 His age was 2 years and 2 months, and he was admitted probably within 20 minutes after he had consumed 5 tablets each containing 0 4 mg LSD A gastric lavage was immediately performed on arrival No remains of the tablets could be identified

On admission the boy was in a state of extreme excitability He also had mydriasis and pronounced ataxia He was unable to walk or grasp in proper manner His respiration pulse heart rate and blood pressure were normal

About 10 minutes later i.e. about 1 hour after the intake of LSD he had reached a state of total catatonia. No spontaneous movements were noted. His body and extremities rigidly remained in the position, in which they were placed. At the same time the boy had become completely lost in himself without loosing consciousness. He had no contact with his surroundings. The mydriasis quickly became more pronounced, and his eyes were kept absolutely fixed. No reflexes could be elicited. He responded only to strong tactile stimuli and strong pain His face was peaceful and showed no signs of agony About 1 / hours after the intake of LSD his respiration became faster and irregular Tachycardia was also noted at this time and his blood pressure had risen to 120 mmHg systolic.

Four hours after the consumption of the LSD tablets, spontaneous movements of the extremities and eyes returned, but there was a marked stiffness and resistance to passive movements of his limbs. The tendon reflexes, however were still absent. The respiration became normal 5 hours after LSD intake. After 6 hours *he fell asleep. During the following hours he slept* peacefully except when the pulse and blood pressure were recorded. After 9 hours he had a 20-minute attack of repeated small convulsions in his extremities. Thereafter a continuous and steady normalization took place. The resistance to passive movements of the limbs, however persisted for 16 hours and it was not until 48 hours after the LSD intake, that his behaviour general condition and neurological status had become completely normal.

Laboratory investigations during the acute phase

Except for a slightly low sodium concentration (131 mEq/l) the serum electrolytes were normal. The acid base balance of the blood, all routine haematological and urinary analyses as well as serum creatinine and

transaminase determinations were normal. An electrocardiogram and two electroencephalograms performed at 3 and 6 days after the intake of LSD were also normal Blood and urine analyses for LSD were negative No methods for the determination of LSD metabolites were available

Treatment

After the gastric lavage the boy was placed in an isolation room, with subdued lighting Reassuring talks were given repeatedly by nurses and doctors during the first 48 hours Because of the small convulsions 10 mg of chlorpromazine was given once only 9 hours after the LSD intake but had no obvious effect on the convulsions

Follow up

The boy was re-examined at the Outpatient Department on two occasions, the last time 1 ½ months after the discharge from hospital

According to the parents the boy had recovered completely and had behaved normally all the time No signs of disease or sequelae could be demonstrated. An electroencephalogram 45 days after the intake was normal. No chromosomal aberrations were found His developmental age was normal with a motor age of 24 months and a Denver test corresponding to 30 months of age His general behaviour seemed normal in all respects

DISCUSSION

There are only a few reports in the literature of the effect of LSD on children (1 2 4 5 6) Most of these are concerned with severely psychotic children in whom LSD had been used in low doses for therapeutic reasons

One reported case of acute intoxication (6) illustrates the acute clinical picture and the risk of late untoward effects after a relatively large dose of LSD A healthy 5 year-old girl had taken 0 1 mg of LSD on one occasion and became acutely psychotic within 20 minutes with agitation panic depression flattening of affect disorientation feelings of depersonalisation distortion of body image and depression of intellectual function She also displayed evidence of organic brain dysfunction with impaired visual-motor and visual-perceptual functions and abnormal electroencephalograms which showed diffuse high voltage slowing and dysrythmia.

In Assmus & Reimer s report (1) of accidental LSD-intoxication in three siblings the acute reaction was not very alarming and subsided in 24 hours but there was a delayed reaction the so-called flashback" Five weeks after the ingestion two of the children again became acutely psychotic and the clinical picture was now more serious and besides the hallucinations they showed a state of great panic and agony

Also well documented are different kinds of permanent chromosomal changes (7) mainly an increased incidence of chromosomal breaks which obviously may appear even after low doses

The lethal dose of LSD seems to be unknown According to Moeschlin the lethal dose greatly varies from person to person Therefore it is difficult to assess if the boy s life was threatened in this case The clinical picture however was alarming which is not surprising in relation to the very high dose ingested (2 mg LSD or 0 18 mg/kg body weight) Despite this rather high dose no long lasting or permanent sequelae seem to have occurred

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BOOK REVIEWS

Margaret A. Lennox-Buchthal *Febrile convulsions. A reappraisal.* Thesis, Institute of Neurophysiology University of Copenhagen, June 1973 138 pp., illus. Electroencephalography and Clinical Neurophysiology Suppl No 32. Elsevier Scientific Publishing Company Amsterdam, London, New York 1973 Price not given.

This book is a very thorough study on febrile convulsions in childhood, based on an extensive literature study and the author's own vast experience. It is indisputedly so that more children with febrile convulsions develop epilepsy than do children without febrile convulsions Theories on the pathophysiology especially in cases developing epilepsy are discussed in detail Is it epilepsy from the beginning or is the epilepsy the result of a brain-damage caused by the febrile convulsions? Maybe both mechanisms are at work. The arguments are sometimes difficult to follow perhaps because there are still many unsettled points. Most of the readers, I think, will find it undesirable to solve the problems as the author did with continuous phenobarbital therapy to all children with febrile convulsions. However the book is highly recommendable, it widens the knowledge on febrile convulsions and gives a comprehensive literature list on the subject.

Ingrid Bjerre

Marcel Bessis. *Living blood cells and their ultrastructure* Springer Verlag, Berlin, Heidelberg and New York 1973 767 pp illus. DM 151 –

During the last few years several books on blood, bone marrow and lymphnode ultrastructure have appeared. Such works are often of a specialized character and chiefly of interest to electron microscopists. The present work by Marcel Bessis, professor of haematology in Paris and well-known to all interested in haematological morphology has much to offer to every general haematologist, though primarily concerned with the appearance of blood cells as seen through the electron microscope Both the classical transmission electron microscope and the newer technique of scanning electron microscopy have been extensively used for the excellent illustrations. The scanning microscope is of particular value in haematology as high resolution, three-dimensional pictures can be produced and this has proved to be of importance especially in research on erythrocyte shape a field where professor Bessis is the acknowledged master Also many illustrations of living blood cells as seen through the phase contrast microscope are provided for comparison and also somewhat sparsely light microscopy pictures of cells stained with the classical techniques of haemato-

The book comprises 767 pages and the space is about equally divided between illustrations and text. An introduction with general comments on structure and function of blood cells is given. Stem cell theories and problems of haematopoietic regulation are briefly reviewed A short description of normal ultrastructural cytology is given, useful to those still not familiar with electron micrographs After this, there are descriptions of normal and pathological morphology of the erythrocytic, granulocytic thrombocytic and lymphocytic series Chapters on monocytes, plasma cells mast cells and the reticulohistiocytic system are included too The chapter dealing with red cells is the most extensive one and includes many illustrations of abnormal erythrocyte shapes This subject has recently been treated by professor Bessis and others in a somewhat more detailed monograph. A long chapter is devoted to haematological malignancies. Finally a description of methods of preparation of blood cells for the various microscopic techniques is given. After each chapter there is an extensive list of references altogether about 5 000 generally covering the subject up to 1972. An adequate subject index is also included.

The illustrations are well chosen and of flawless quality The magnification of the electron micrographs is generally given as a figure in the legend This tends to make it meaningless to the general reader A reference bar in the picture is preferable Some pictures lack information on magnification altogether

The nomenclature of cells and diseases is well in accord with generally accepted systems in English literature except perhaps in the case of the malignant lymphomas.

The text deals primarily with morphology but discussions of blood cell physiology are also given in many places.

The chapter on techniques is lengthy but it seems doubtful if anyone starting to work with the methods described will find enough information with this book as a manual. The many references are valuable however

The price does not seem to be excessive with regard to the quality of printing and illustrations. The book is recommended to workers in morphological haematology It contains a wealth of information however often difficult to find in other ways, making it of value also to the general haematologist.

Alf Rausing

I. Kolvin, R. C. MacKeith & S R. Meadow (eds.): *Bladder control and enuresis* Clinics in Developmental Medicine Nos. 48/49 William Heinemann Medical Books Ltd., London 1973 320 pp., illus. £6.20

Enuresis in children is a comprehensive therapeutical problem This book which derives from an International Colloquium supported by Geigy Pharmaceuticals gives an extensive review of the contributing factors in a multifactorially determined complaint It provides a board basis for therapy and a reminder that the treatment of enuresis does not always consist in dealing with the bladder or any underlying contributory anomaly but that full attention must also be paid to psychological aspects

The book also gives a summary of the development of bladder control behaviour and an interesting survey of the epidemiology and crosscultural aspects of enuresis The description is completed with a section on indications for future research

Some criticism however has to be presented

In a book so spaciously written, it would have been worth while mentioning that a history revealing postinfectious secondary enuresis can be the consequence of residual urine due to vesico-ureteric reflux Such a suspicion constitutes an indication for a thorough radiological examination Whilst awaiting disappearance of the reflux and thus of the residual urine double or triple micturition before going to bed may be helpful

Although tricyclic antidepressants often are effective in the treatment of enuresis they are not the only drugs convincingly proved to be better than a placebo The efficiency of a *combination* of a parasympathicolytic *and* a sympathicomimetic such as a propantheline and ephedrine ought to have been taken into consideration.

Amphetamine treatment must be considered reprehensible The authors have tried to avoid repetition by re writing the papers from the colloquium but have not been entirely successful The conclusions from Rutter's Isle-of Wight study are referred to in many of the chapters and it hardly requires therefore a chapter of its own The extensive reference lists accompanying almost every chapter are also to a great extent repetitions. It would have been preferable to have all the references presented together at the end of the book.

In my opinion the section on bell and pad treatment is too extensive

As a survey of a common complaint with various and often hypothetical explanations and differing therapeutical approaches the book can be recommended not only to paediatricians but also to psychiatrists and psychologists caring for children with urinary incontinence whether this is a developmental disorder" or a "maturational delay It offers little new however to those who are perpetually busy analysing and treating enuresis

Ingemar Helin

Acta Pædiatr Scand 63 801–804 1974

CYSTINE A SEMI ESSENTIAL AMINO ACID IN THE NEWBORN INFANT

F POHLANDT

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ABSTRACT Pohlandt, F (Department of Paediatrics, Section of Neonatology University Hospital, Ulm, Federal Republic Germany). Cystine: a semi-essential amino acid in the newborn infant. Acta Paediatr Scand 63 801 1974.—The enzyme activities of the transsulfuration pathway (Cystine biosynthesis) are low or not measurable in the livers of human fetuses, premature and full-term newborns. Cystine thus may be an essential amino acid in newborn infants. To test this suggestion we studied the plasma amino acid concentrations in 20 premature and 7 full-term infants treated for prematurity and/or respiratory distress syndrome by ion exchange columnchromatography The infants received infusions of a 6% glucose-electrolyte-solution during the first two days of life. In premature newborns the plasma cystine concentrations markedly decreased within the first 12 hours of life (median <5 μmol/l) and remained low thereafter In full-term infants the plasma cystine concentrations decreased similarly To rule out the possibility that these low plasma cystine concentrations were the result of a reduced cystine biosynthesis due to lack of methionine a substrate of transsulfuration pathway we studied prematures on infusion of a mixture of synthetic *l*-amino acids free from cystine. Despite elevated plasma methionine concentrations (median 113.4 μmol/l) the plasma cystine concentrations remained very low Cystine therefore, can be considered a semi-essential amino acid in the newborn infant and should be supplied to newborns receiving balanced parenteral nutrition.

KEY WORDS: Newborn infants, cystine, essential amino acids, parenteral nutrition

Until now cystine was not considered an essential amino acid for man because in the adult cystine is thought to be synthesized in adequate amounts from methionine via the transsulfuration pathway (3 4) Recently Sturman et al and Gaull et al examined the activities of some transsulfuration pathway enzymes during intrauterine development. The authors found in liver and brain of fetuses and premature newborns a low ered activity of methionine activating enzyme and of cystathionine synthase and lack of cystathionase activity (2, 9) Cystine therefore appeared to be an essential amino acid in the liver and brain of immature newborns

Snyderman (5) observed an impaired weight gain and nitrogen retention in premature infants when cystine was removed from the diet. However details about the composition of formula were not reported

Stegink & Baker (7) and Wei et al (10) found decreased plasma cystine concentrations in newborns and young infants during infusion of a cystine free casein hydrolysate.

Recently Stegink & Den Besten (8) demonstrated that plasma cystine concentration decreased markedly in healthy adults alimentated with casein hydrolysate solutions free from cystine but rich in methionine

Enuresis in children is a comprehensive therapeutical problem. This book which derives from an International Colloquium supported by Geigy Pharmaceuticals gives an extensive review of the contributing factors in a multifactorially determined complaint. It provides a board basis for therapy and a reminder that the treatment of enuresis does not always consist in dealing with the bladder or any underlying contributory anomaly but that full attention must also be paid to psychological aspects

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Although tricyclic antidepressants often are effective in the treatment of enuresis they are not the only drugs convincingly proved to be better than a placebo The efficiency of a *combination* of a parasympathicolytic *and* a sympathicomimetic such as a propantheline and ephedrine ought to have been taken into consideration.

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Ingemar Helin

Table 1 *Amino acid composition of synthetic l amino acid solution Aminofusin® L forte (mmoles/l)*

L Threonine	16 8
L Proline	52 1
Glycine	333 0
L Alanine	291 8
L Valine	77 3
L Methionine	32.2
L Isoleucine	71 3
L Leucine	36 6
L Phenylalanine	76 6
L Histidine	19 4
L Lysine	27 4
L Tryptophan	4 9
L Arginine	74 9

These observations suggest that even in the adult the synthesis of cystine from methionine is limited during parenteral nutrition

The present communication reports on the

(*a*) plasma cystine concentrations in newborn infants receiving proteinfree parenteral nutrition and

(*b*) on the influence of a high methionine supply on plasma cystine concentrations in premature infants receiving infusions of a cystine free mixture of synthetic *l* amino acids

METHODS

Subjects

Twenty premature and seven full-term newborns who were treated for prematurity and/or respiratory distress syndrome receiving infusions of a 5% glucose-electrolyte solution during the first two days of life (rate of infusion 100–150 ml/kg bodyweight/day) No oral feedings were given Plasma samples were obtained repeatedly in about 6 hour intervals from an umbilical artery catheter or by venous puncture

Six premature infants who were treated for prematurity and/or respiratory distress syndrome and received no oral feedings were infused of a mixture of synthetic *l*-amino acids carbohydrates and electrolytes starting on the second day of life (rate of infusion 100–150 ml/kg bodyweight/day corresponding to 3–4 g amino acid/kg bodyweight/day Aminofusin® L forte[1] composition see Table 1) Twelve to twenty-four hours after starting the infusion plasma samples were obtained from an umbilical artery catheter or venous puncture

Amino acid determinations

To avoid losses of cystine blood samples were centrifugated immediately The plasma was deproteinized with four times the same volume of 3% aqueous solution of sulfosalicylic acid (1) The supernatants were stored at −25°C and analyzed by modifications of the method of Spackman et al (6) in a Biotronik[2] amino acid analyzer Column size 58×0 9 cm Resin Aminex A5[3] First buffer 0 14 N sodium citrate pH 3 4 second buffer 0 2 N sodium citrate pH 3 78 third buffer 0 46 N sodium citrate pH 3 86 T_1 30°C T_2 62°C First buffer and temperature change after 175 min during appearance of valine buffer flow 70 ml/hour Using the amino acid calibration standard Type P AN the coefficient of variation came to 3 7% for cystine (n=11) and 3 0% for methionine (n=9) Due to small sample volumes (0 04–0 16 ml plasma) this method had a lower limit of sensitivity at 5 µmoles cystine/l plasma

RESULTS

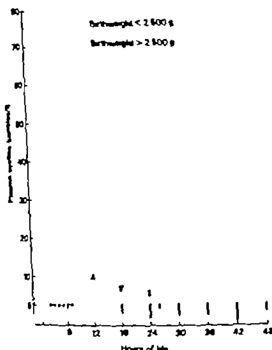
The plasma cystine concentrations of newborns receiving infusions of a glucose-electrolyte solution are shown in Fig 1 and reported in Table 2 The cystine concentration of premature infants dropped markedly within the first twelve hours of life (median <5 µmoles/l) and remained low until the second day of life In full term newborns the plasma cystine concentrations decreased similarly In seven of twenty plasma samples obtained from seven full term newborns during the first two days of life cystine concentration was lower than 5 µmoles/l

The plasma cystine concentrations of six premature infants receiving infusions of a mixture of synthetic *l*-amino acids free from cystine are shown in Table 3 Inspite of elevated plasma methionine concentrations the cystine concentrations remained depressed

DISCUSSION

Simultaneous to the drop of plasma cystine concentrations most amino acids decreased in newborns receiving no protein Depressed plasma cystine concentrations therefore could be the result of a reduced cystine bio-

[1] J Pfrimmer+Co Erlangen
[2] Biotronik Wissenschaftliche Geräte GmbH Frankfurt/Main
[3] Bio Rad Laboratories, Richmond California
[4] Hamilton Co Whittier California



1 Cystine plasma concentrations in newborn in-
s receiving infusions of 5% glucose-electrolyte-solu-
during the first two days of life

thesis due to a lack of methionine a sub-
ate of the transsulfuration pathway How
er this possibility was ruled out in pre
ture infants receiving a cystine free but
ethionine rich mixture of synthetic *l* amino
ids: the cystine concentrations remained
w despite elevated plasma methionine con-
ntrations

Equal amounts of amino acids will reach higher concentrations in portal venous blood on oral feeding route than during parenteral administration Thus the biosynthetic rate of reactions in liver amino acid metabolism dependent on substrate could be decreased during parenteral nutrition Our data (Table 3) however indicate that the cystine biosynthesis is quite limited in newborns independent of substrate concentration

In the plasma of all newborns studied we found cystathionine up to 40 µmol/l corresponding to the lack or very low activities of cystathionase in immature and full term newborns (2 9)

Our data indicate that in the premature infant cystine is a semi-essential amino acid The similar decrease of plasma cystine in both premature and full-term newborns suggests that cystine is a semi-essential amino acid in full-term newborns as well Consequently we suggest that newborns receiving balanced parenteral nutrition should be supplied with cystine in order to maintain a normal plasma cystine concentration (55 µmol/l) (1 8) until we know the lowest possible level of plasma cystine compatible with normal growth in newborns Preliminary results demonstrated that 80 mg cysteine/kg bodyweight/day was sufficient to obtain normal plasma cystine concentrations in newborns on parenteral nutrition.

Table 2. *Cystine plasma concentrations in premature newborns receiving infusions of a % glucose-electrolyte solution*

	Hours of life								
	0	1–6	7–12	13–18	19–24	25–30	31–36	37–42	43–48
n	2	11	8	10	13	10	7	8	4
Mean									
Birthweight (g)	1 790	1 711	1 585	1 555	1 525	1 587	1 710	1 625	1 493
±S E M		107	121	149	118	138	177	175	
Cystine (µmoles/l)									
Median		22.6	<5	6 8	5.0	<5	<5	<5	<5
Range									
from	30 0	<5	<5	<5	<5	<5	<5	<5	<5
to	45.6	74 8	10 4	44.3	28 4	13.4	20 1	19.3	5.2

Table 3 *Cystine plasma concentrations during the 37–48 hour of life in premature newborns receiving various infusions*

	Group I	Group II*	Normal (1 8) values
n	8	6	
Mean birthweight (g)	1 639	1 772	
±S E.M	169	87	
Cystine (μmoles/l)			
Median	<5	8 ?	55
Range			
from	<5	<5	
to	19.3	19 4	
Methionine (μmoles/l)			
Median		113 4	30
Range			
from		40 1	
to		??0 6	

Prematures receiving infusions of 5% glucose-electrolyte solution
* Prematures receiving infusions of a cystine free mixture of synthetic *l*-amino acids (3–4 g amino acids/kg bodyweight/day) carbohydrates and electrolytes

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PROTEOLYTIC ACTIVITY IN DUODENAL JUICE IN INFANTS CHILDREN AND ADULTS

TOR LINDBERG

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ABSTRACT Lindberg T (Department of Paediatrics, Malmö General Hospital, Malmö Sweden). Proteolytic activity in duodenal juice in infants, children, and adults. Acta Paediatr Scand 63:805 1974. The capacity of duodenal juice to hydrolyse casein to trichloroacetic acid-soluble peptides was investigated: in a control group (11 infants and children and 7 adults); in 5 children with pancreatic disease; in 10 children with enterogenic malabsorption (coeliac disease and cow's milk protein intolerance). The proteolytic activity per ml duodenal juice in fasting condition was as great in healthy infants and children as in healthy adults (500–600 mg casein/ml/hour). There was good correlation between trypsin content and proteolytic activity of duodenal juice In pancreatic insufficiency the proteolytic activity was extremely low (not measurable in 3 patients) as was the trypsin activity whereas in enterogenic malabsorption, it was within the ranges of the controls.

KEY WORDS: Duodenal juice, proteolytic enzymes, pancreatic function trypsin

As known, 75–80% of dietary protein is digested and absorbed in the upper part of jejunum shortly after ingestion (2, 4 14) Thus the capacity of the gut to utilize protein seems to be high Remarkably however only few studies attempting to quantitate the proteolytic activity in intestinal juice have been performed Goldberg et al (8) in 1968 investigated the proteolytic activity in ileal drainage material with a method they themselves developed (7) They found a very good proteolytic activity theoretically several kilograms of protein could be digested per day They also meaned that an estimation of total proteolytic activity would appear to be the most appropriate test for pancreatic function

The purpose of this investigation was to study the proteolytic activity in duodenal juice in various age groups in healthy individuals In addition some children with pancreatic insufficiency and some with enterogenic malabsorption were studied Comparison was made with the trypsin determination to evaluate the representativeness of this test for the proteolytic capacity *in vivo*

MATERIAL

Controls

The group consists of 11 infants and children (aged: 2.5 4 6 and 12 months, and 1.3 1.5 .., 2.5 3 3.5 and 11 years) and 7 adults. The infants and children were investigated for gastro-intestinal complaints. All showed normal xylose test, faecal fat excretion normal trypsin content in duodenal juice (>130 μg/ml) (3 13) and normal mucosal histology in small intestinal biopsies Two adults were healthy: five had chronic renal failure and were studied for other purposes (5). All had normal trypsin content (13) in duodenal juice.

Pancreatic insufficiency

Four patients, aged 6, 6 8, and 20 months, had cystic fibrosis of the pancreas (all had abnormal sweat test and chronic pulmonary disease) One boy had Shwachman syndrome (exocrine pancreatic insufficiency neutropenia, metaphyseal dysostosis, and dwarfism). He was studied at 9 months and at years 10 months of age All except one 6-month-old boy had clinical

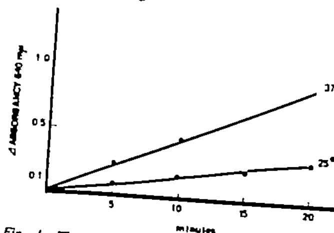


Fig 1 Time curve of total proteolytic activity of duodenal juice at 25°C and 37°C
Δ absorbancy = absorbancy of the test sample minus absorbancy of the time blank

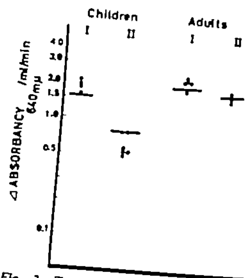


Fig 2 Total proteolytic activity of duodenal expressed as increase of absorbancy at 640 nm and minute in controls (children = infants + chil (one child was studied on two occasions)

and laboratory signs of malabsorption in the form of failure to gain weight and steatorrhoea respectively They did not have any pancreatic enzymes before this study

Enterogenic malabsorption

This group includes 9 children with coeliac disease aged 6 and 8 months, 1 1.5 1.5 2, 2, 3 and 3.5 years. The diagnosis was determined on a flat intestinal mucosa histological and clinical improvement on gluten-free diet and histological and/or clinical relapse on gluten-containing diet.

Finally a girl aged 3 months with a cow s milk protein intolerance was studied. She reacted with vomiting and diarrhoea on two occasions when exposed to cow s milk. All patients in this group had normal trypsin content in duodenal juice.

In short this method with the aid of Folin Cioca reagent measures the acid-soluble peptides for during incubation of duodenal juice (diluted 1 m 30) with a casein–phosphate buffer solution (Hamm sten casein BDH) Fig. 1 shows a time curve of s a hydrolyse at 25°C and 37°C expressed as increase absorbancy The obtained values can then be conver to mg casein digested per ml juice/min by using a ca bration curve from acid-hydrolysed casein as describ by Goldberg McAllister & Roy (8)

Trypsin activity was determined with α-N benzoy DL-arginine-p-nitroanilide HCl (BAPNA) (Sigma) a substrate (6 12 13)

METHODS

Duodenal juice was collected via a double lumen tube (Salem sump tube (Sherridan)) from the transversal or ascending part of duodenum (fluoroscopic control) Two fractions were collected one fasting fraction (in infants 6 hours fasting In children and adults over night) and one up to 60 minutes after a test meal consisting of water (100 to 300 ml depending on age) (1) However 2 children in each of the three groups received 100 ml of breast milk or a formula diet instead of water In agreement with the results of Bergström & Lund (1) there was no significant difference of trypsin content in duodenal juice between those receiving water and those receiving breast milk or formula diet

The juice was collected in crushed ice and analysed either directly or after storage at –20°C The proteolytic activity was found to be stable under those conditions (cf 7)

Total proteolytic activity (TPA)

The proteolytic activity was determined as described by Goldberg McAllister & Roy (7 8) except that the incubation was performed at 37°C instead of 25°C

RESULTS

Fig 2 shows the individual values of the total proteolytic activity (TPA) of the duodenal juice in the controls before and after the test meal TPA is here expressed as the increase in absorbancy per ml/min As seen TPA in fasting condition was of the same magnitude in children as in adults but the ranges were greater in the children There was no correlation between age and TPA the youngest infant had the second highest value After the test meal (fraction II) the TPA was lower in the children than in the adults This could well be due to a relatively greater dilution of duodenal juice by the test meal in children than in adults Table 1 reports the obtained mean values and ranges of TPA in children and adults converted to mg casein digested per ml duodenal juice/hour

Table 1 *Amount of casein hydrolysed to acid soluble peptides by duodenal juice in the controls (mg/ml/hour: mean values and ranges)*

	Infants and Children	Adults
Fasting	900 (50–960) (n = 12)	600 (370–750) (n = 7)
After test meal	250 (50–660) (n = 10)	540 (420–650) (n = 6)

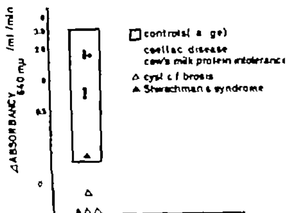


Fig 4 Total proteolytic activity in pancreatic insufficiency and enterogenic malabsorption.

There was a good correlation between the trypsin activity and the TPA in the same sample of duodenal juice (r = 0 68) (Fig 3)

Fig. 4 shows the TPA in fasting condition in the two groups of children suffering from malabsorption disorders The children with enterogenic malabsorption (coeliac disease and cow's milk protein intolerance) all had TPA within the ranges of the controls In the pancreatic insufficiency group 3 children had zero values of TPA (and of trypsin) in the fasting condition and after the test meal One child with a low but measurable TPA had also a low trypsin content in duodenal juice (47 μg/ml) These 4 patients had clinical signs of malabsorption and had steatorrhoea. The child with Shwachman's syndrome was studied twice with the same result The fifth child who had cystic fibrosis of the pancreas had normal weight gain and normal stools He had a normal pancreatic function as judged by the trypsin content in duodenal juice (190 μg/ml) and the TPA was just above the lower range of the controls

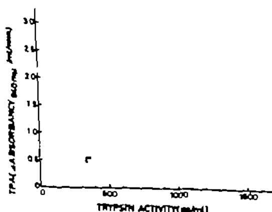


Fig 3 Total proteolytic activity (TPA) correlated to trypsin activity in the same sample of duodenal juice (r = 0 68)

DISCUSSION

The study has shown that the capacity of the duodenal juice to digest casein to acid-soluble peptides is as great in infancy and childhood as in adults This finding agrees with the knowledge that the proteolytic enzymes from pancreas are well developed in early life and do not increase with age (9)

The design of the present investigation—without quantitative collection of the duodenal juice—does not allow calculation of the absolute amount of casein that can be hydrolysed by the duodenal juice to acid soluble peptides per hour If however the figures for volume of duodenal juice reported by Hadorn et al (9) for children (about 4 ml/kg body weight/hour) and by Hartley et al (10) and Rosenberg et al (15) for adults (about 3 ml/kg body weight/hour) are used the following (total) values are obtained In adults using the figures obtained after the test meal (see Table 1) 1 6 g casein

could be digested per kg body weight and hour This value is of about the same magnitude as that reported by Goldberg et al (8) In children the corresponding value will be 1 0 g This means that the duodenal juice in an infant 5 months of age and weighing 7 kg can digest about 7 g casein per hour (equal to about 250 ml cow s milk) This value can be compared with the results of Hiratha et al (11) obtained in intestinal intubation studies They collected intestinal juice from ileum and analysed it for casein after feeding infants of various ages a formula with varying amounts of cow s milk protein They found an increasing ability to digest and absorb casein with increasing age e g an infant aged 5 months could completely utilize the casein in a meal of about 200 ml (150 ml/kg/day) containing 2 5% milk protein but not 2 7%

From the present study it can be concluded that the relatively limited ability to digest casein in infancy is not due to a lower proteolytic activity in the duodenal juice *per se* but to its smaller total volume In this context it should be mentioned that in the present study a possible effect of gastric juice has not been considered

The TPA in duodenal juice is the (total) result of the action of several proteolytic enzymes of pancreatic origin such as trypsin chymotrypsin elastase and carboxypeptidase and therefore better reflects the protein digestion *in vivo* than single enzyme measurements Goldberg et al (8) found that about two-thirds of TPA was due to the action of trypsin and chymotrypsin Consistently it was found that in pancreatic insufficiency the TPA was very low—as was the trypsin activity In enterogenic malabsorption on the other hand the TPA was within the ranges of the controls However the assay method of TPA is more laborious than that of trypsin As there is a good correlation between the TPA and the trypsin content measurement of trypsin activity is therefore preferable for routine clinical use

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CHLORHEXIDINE TREATMENT OF ORAL CANDIDIASIS IN SERIOUSLY DISEASED CHILDREN

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ABSTRACT Langslet, A., Olsen, I., Lie, S. O and Løkken, P (Department of Paediatrics, Rikshospitalet, and the Dental Faculty University of Oslo, Norway). Chlorhexidine treatment of oral candidiasis in seriously diseased children. Acta Paediatr Scand 63 809 1974.—Chlorhexidine, a broad-spectrum antibacterial substance with antifungal activity was tested in 8 children with acute oral pseudomembranous candidiasis (thrush) and in one child with chronic mucocutaneous candidiasis. Five of the patients suffered from acute leukaemia. The drug was administered in a 0.2% solution, either as mouth rinses or painted on the lesions, for 6–17 days for those having the acute form and for 90 days in the case with chronic candidiasis. All patients with thrush were clinically cured and in the 6 cases where mycological examination of oral swabs was undertaken before and after therapy the cultures became negative. Like a number of antimycotics, chlorhexidine failed to cure the oral lesions in the patient with chronic mucocutaneous candidiasis. No adverse reactions attributable to the drug were observed. The results obtained in this limited number of patients are promising, and in oral fungal infections chlorhexidine deserves further attention as an alternative to drugs like gentian violet, nystatin or amphotericin B. It is suggested that prophylactic use of chlorhexidine may be justified to obtain and maintain the best possible oral health in seriously diseased patients.

KEY WORDS: Chlorhexidine, oral candidiasis, leukaemia

Chlorhexidine has recently received much attention in dentistry as it has been shown to be able to inhibit the development of dental plaque gingivitis supra-gingival calculus and smooth surface caries (9 14). It may thereby represent a valuable addition/alternative to mechanical methods in oral hygiene

Most studies in oral medicine have been concerned with the antibacterial effects of chlorhexidine (14) but its usefulness in candida-induced denture stomatitis has also been demonstrated (2) We therefore considered it worthwhile to undertake a pilot trial on the therapeutic effects of chlorhexidine in oral candidiasis in children

MATERIAL AND METHODS

Eight children, aged 2 13 years, with the clinical characteristics of acute oral pseudomembranous candidiasis (thrush) and one child of 7 years with chronic mucocutaneous candidiasis, were treated with 0 2% chlorhexidine solution (Hibitane® I C I chlorhexidine digluconate 20% diluted with distilled water) With patients not capable of performing mouth rinses, the solution was painted on the oral lesions, while others rinsed the mouth with 10 ml volumes from –6 times daily As seen in Table 1 all children were suffering from serious diseases most of them receiving drugs which may favour the occurrence of fungal infections. In all but patients, smears were taken for yeast culture on Sabouraud s dextrose agar

RESULTS

The results are summarized in Table 1 In all cases of thrush the lesions began to heal within 2–4 days Those able to communicate reported reduced discomfort 24–48 hours after the therapy had started. Within 6–17 days the lesions disappeared the symptoms having cleared up completely 1–2 days beforehand

Table 1 *The effect of local application of 0.2 % chlorhexidine solution in oral candidiasis*

Case no	Sex	Age (years)	Diseases	Drugs	Candidiasis form	Chlorhexidine treatment: Procedure	Chlorhexidine treatment: Times daily
1	M	7	Acute myeloblastic leukemia	Cytostatics	Thrush	Mouth rinses	
2	M	13	Acute myeloblastic leukemia bone marrow aplasia	Cytostatics antibiotics	Thrush	Mouth rinses	4
3	M	11	Acute lymphoblastic leukemia	Cytostatics antibiotics	Thrush	Mouth rinses	6
4	F	8	Acute lymphoblastic leukemia	Cytostatics	Thrush	Mouth rinses	6
5	F		Acute lymphoblastic leukemia	Cytostatics prednisone antibiotics	Thrush	Paintings	6
6	F	2	Chronic hepatitis with hepatic decompensation	–	Thrush	Paintings	6
7	F	11	Chronic meningo-encephalitis (?) grand mal epilepsy	ACTH	Thrush	Paintings	6
8	F	10	Wissler's syndrome pericarditis with effusion heart failure	Prednisone antibiotics	Thrush	Mouth rinses	3
9	M	7	Hypoparathyroidism hypoadrenocorticism	–	Chronic mucocutaneous	Mouth	2

From 6 of these children oral swabs for yeast culture were taken before and after treatment At the end of the treatment no *Candida albicans* was found

In case 9 (the 7 year-old boy with chronic mucocutaneous candidiasis) chlorhexidine mouth rinses twice daily for 3 months failed to give any effect

No adverse effects attributable to chlorhexidine were observed

DISCUSSION

Although oral candidiasis is often regarded as a minor ailment it may be most distressing to certain patients and may affect food intake and general health There is furthermore a risk of dissemination of the infection This particularly applies in cases with impaired defence mechanisms like those in the present series A recent retrospective study on 65 consecutive patients who died of acute leukemia showed that 28% had severe fungal infections at autopsy the gastrointestinal tract and the lung being the organs mo frequently involved (12)

A report by Faber & Dickey in 1925 (made application of gentian violet tł standard therapy in thrush with clinic success rates quoted to about 70% in th literature (4) Although relatively effective the dye stains tissues and clothing and ma cause superficial necrosis (the gentian violet burn) and thus aggravate the condition (3) Higher success rates are obtained with antifungal antibiotics like nystatin and amphotericin B but even these drugs do not cure all cases of thrush (1 4 11) and they have been claimed to have little effect when the patients general immune responses are impaired such as in acute leukemia (10)

The results obtained with chlorhexidine in the present pilot trial are encouraging as all children with oral thrush were cured Chlorhexidine proved to be efficient in cases where gentian violet (0 5%) had failed The poor response in the boy with chronic mucocutaneous candidiasis was not unexpected

Days before complete disappearance of oral lesions	Yeast culture	
	Before treatment	After treatment
6	–	
6		–
7	Pos.	Neg
10	Pos.	Neg
14	Pos	Neg
7	Pos.	Neg
17	Pos	Neg
14	Pos	Neg
No improvement	Pos	Pos

since it is a remarkable feature of this type of infection that it is extremely resistant to all kinds of topical therapy (8). Actually a number of antifungal drugs (gentian violet nystatin amphotericin B and clotrimazole) had been tried in this case without success.

Chlorhexidine has been commonly used as an antiseptic since 1953. Both animal tests and long term clinical experience indicate a low level of toxicity and less than 10 cases of sensitization have been recorded (7). Studies with 0.2% chlorhexidine mouth rinses twice daily in humans for up to 2 years indicate that only slight and most likely inconspicuous changes take place in the susceptibility of the oral microorganisms (13). A noticeable side effect of chlorhexidine is the yellow-brown staining of the teeth and the dorsum of the tongue which in some patients may occur after a few days of mouth rinsing (6).

The ability of chlorhexidine to prevent bacterial colonization on teeth is well documented (14) and as demonstrated in the present work the drug may also be of value in oral fungal infections. We consider that in selected cases even prophylactic use at least intermittently may be justified. In seriously diseased patients like those presently treated improvement of the oral conditions may diminish their suffering and prevent the spread of serious infections from the oral cavity.

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THE EFFECT OF L DOPA ON THE BLOOD CONCENTRATIONS OF GROWTH HORMONE THYROTROPHIN GONADOTROPHINS CORTISOL AND GLUCOSE IN CHILDREN WITH SHORT STATURE

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ABSTRACT Nilsson K O and Thorell J I (Department of Paediatrics, Endocrino-logy and Nuclear Medicine University Clinics, Malmö General Hospital Malmö, Swe-den) The effect of L-dopa on the blood concentrations of growth hormone thyro-trophin gonadotrophins, cortisol and glucose in children with short stature. Acta Pae-diatr Scand 63 812, 1974.—Studies were performed in nine constitutionally short children to evaluate the diagnostic usefulness of oral L-dopa as compared to insulin induced hypoglycemia as a provocative agent for the stimulation of growth hormone production In addition the effect of L-dopa on the blood concentrations of thyro-trophin gonadotrophins, cortisol and glucose was investigated The mean growth hor mone concentration was 4.8±1.6 (S.E.M) ng/ml before L-dopa and 2.9±1 0 ng/ml be-fore insulin. The mean peak growth hormone concentration was 20.7±2.6 ng/ml after L-dopa and 17.5±3 1 ng/ml during insulin induced hypoglycemia. All children showed peak growth hormone concentrations at or above 8 ng/ml both after L-dopa and insulin, although in one patient this level was reached only after priming with testosterone No significant changes in the blood concentrations of thyrotrophin gonadotrophins, cortisol or glucose were observed It is concluded that L-dopa is an effective stimulus for the release of growth hormone in young subjects.

KEY WORDS: L-dopa anterior pituitary hormones

Of the many procedures available to test growth hormone (GH) reserve (10) insulin in duced hypoglycemia is probably the most common (9) This test however is connected with potentially dangerous side effects such as convulsive seizures Accordingly it is desirable that the clinical usefulness of other GH stimulatory procedures be tested In view of the reports of Boyd et al (4) and others (7 14) that L-dopa can stimulate GH production in adult subjects we have com pared the diagnostic usefulness of oral L dopa with that of insulin induced hypo-glycemia in man In addition we investigated the effect of L-dopa on the plasma levels of thyrotrophin (TSH) follicle stimulating hor mone (FSH) and luteinizing hormone (LH) Serum cortisol and blood sugar were also followed

MATERIAL AND METHODS

Nine children aged 6–16 years, were studied (Table 1 All were considered to have constitutional short statu on basis of history physical examination bone age X rays and standard pituitary evaluation The tests wit insulin hypoglycemia and L-dopa were performed wit an interval of at least three days All tests were starte in the morning after overnight fasting and under ab solute bed rest Blood samples were drawn via a cannul (Venflon Viggo) which was inserted in a peripheral vein 30 minutes prior to the test. A single oral dose of L-dopa (Larodopa® Roche) was given (700–500 mg) the dosage depending on the weight of each subject (Table 1) Crystalline insulin was given intravenously

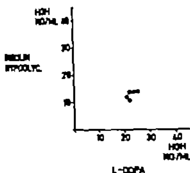


Fig. 1 Peak growth hormone values following L-dopa plotted against those following insulin hypoglycemia

0.075–0.1 u/kg body weight) and was followed by a decrease in blood sugar with more than 50 per cent within less than 30 minutes. The cannula was kept open by a slow infusion of physiological saline

Blood samples were collected in tubes with EDTA for the determination of GH TSH FSH and LH every 30 minutes for 180 minutes. After centrifugation the plasma was frozen for later analysis. With the same intervals blood was also taken for the determination of serum cortisol and blood sugar One of the patients (L. J) was evaluated before and after priming with testosterone propionate (Testoviron® Schering AG), 25 mg i.m. daily for 4 days.

GH, TSH, FSH and LH were determined in triplicates with radioimmunoassay utilizing double antibody systems for the isolation of antibody bound radioactivity (16, 20, 21 25). The coefficients of variation of the means of triplicates were always less than 5% An elevation of GH to at least 8 ng per milliliter was considered to be a normal GH response Cortisol was determined using a modification of the fluorometric procedure described by de Moor et al (18). Glucose was measured with a glucose oxidase method according to Hjelm & de Verdier (11).

RESULTS

The effect of L-dopa and insulin hypoglycemia on plasma GH concentration is shown in Table 2 The mean basal GH concentration was 4 8±1 6 (S E.M) ng/ml before L-dopa and 2.9±1 0 ng/ml before insulin, which values were not significantly different ($p>0.3$ paired t-test) GH concentration increased significantly over baseline both after L-dopa and insulin ($p<0.001$ paired t-test) and all nine children showed peak GH concentration at or above 8 ng per milliliter although in one patient this level was achieved only after priming with testosterone.

The mean peak GH concentration was 20 7 ±2 6 ng/ml after L-dopa and 17.5±3 1 ng/ml during insulin induced hypoglycemia The peak GH response to L-dopa was not significantly different from that following hypoglycemia ($p>0.3$ paired t test) In Fig. 1 the peak GH values following L-dopa are plotted against those following insulin hypoglycemia.

The effect of L-dopa on the plasma levels of TSH FSH and LH serum cortisol and blood sugar is shown in Table 3 No significant changes were observed

Nausea and/or vomiting followed L-dopa in some subjects but there was no obvious correlation between symptoms and plasma GH response (Table 2)

DISCUSSION

In the present studies the GH response to L-dopa was well comparable to that induced by insulin hypoglycemia. While this study was in progress similar results were reported by Root & Russ (23) and Chakmakjian et al (6) Thus there exists good evidence that L-dopa can be used clinically to test the capacity of the pituitary to produce GH L-dopa has the advantage over insulin that the patient is not subjected to the risk of potentially dangerous convulsive seizures L-dopa sometimes causes temporary nausea and vomiting, but these side effects—although unpleasant—

Table 1 *Patient material*

Subject	Sex	Chronologic age (y mo)	Bone age (y mo.)	Per oral L-dopa dose (mg)
1 R. S.	M	6:8	6:8	200
2. C L	F	6:0	5 6	200
3 C. O	M	8 6	8:0	200
4. K. E. J	M	13:6	11:9	400
5 L. J	M	12:8	11:0	400
6. A. M. J	F	12:6	10 6	300
7 B A. R.	M	14 4	11.0	300
8. P H.	F	12:4	9 6	400
9 M. S.	M	15 9	13:9	500

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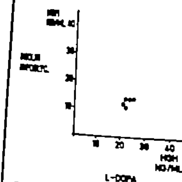


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The effect of L-dopa and insulin hypoglycemia on plasma GH concentration is shown in Table 2 The mean basal GH concentration was 4 8±1 6 (S E.M) ng/ml before L-dopa and 2 9±1 0 ng/ml before insulin which values were not significantly different ($p>0.3$ paired t test) GH concentration increased significantly over baseline both after L-dopa and insulin ($p<0\ 001$ paired t test) and all nine children showed peak GH concentration at or above 8 ng per milliliter although in one patient this level was achieved only after priming with testosterone

The mean peak GH concentration was 20 7 ±2 6 ng/ml after L-dopa and 17.5±3 1 ng/ml during insulin induced hypoglycemia The peak GH response to L-dopa was not significantly different from that following hypoglycemia ($p>0.3$ paired *t* test) In Fig 1 the peak GH values following L-dopa are plotted against those following insulin hypoglycemia

The effect of L-dopa on the plasma levels of TSH FSH and LH serum cortisol and blood sugar is shown in Table 3 No significant changes were observed

Nausea and/or vomiting followed L-dopa in some subjects but there was no obvious correlation between symptoms and plasma GH response (Table 2)

DISCUSSION

In the present studies the GH response to L-dopa was well comparable to that induced by insulin hypoglycemia. While this study was in progress similar results were reported by Root & Russ (23) and Chakmakjian et al (6) Thus there exists good evidence that L-dopa can be used clinically to test the capacity of the pituitary to produce GH L-dopa has the advantage over insulin that the patient is not subjected to the risk of potentially dangerous convulsive seizures L-dopa sometimes causes temporary nausea and vomiting, but these side effects—although unpleasant—

Table 1 *Patient material*

Subject	Sex	Chronologic age (y :mo.)	Bone age (y :mo)	Per oral L-dopa dose (mg)
1 R. S	M	6.8	6-8	200
2. C I	F	6.0	5-6	200
3 C O	M	8 6	8-0	200
4 K. E. J	M	13 6	11-9	400
5 L. J	M	17-8	11-0	400
6. A. M. J	F	12-6	10-6	300
7 B A R.	M	14-4	11-0	300
8. P H.	F	12.4	9.6	400
9 M. S	M	15-9	13-9	300

Table 2 *Plasma levels of growth hormone (ng/ml) at various times after I L-dopa* [cut off at right edge] *and II insulin i v*
For doses see Table 1

Subject	Type of study	Minutes 0	30	60	90	120	180	Side effects
1 R S	I	1	1	8	6	3	3	Nausea
	II	1	13	6	4	4	2	
2 C I	I	1	35	19	9	4	1	Vomiting
	II	5	16	21	17	8	1	
3 C O	I	5	24	18	2	2	1	Vomiting
	II	1	14	6	2	1	1	
4 K E. J	I	17	13	24	14	8	1	
	II	1	40	24	13	7	3	
5 L. J	I	5	7	4	2	1	1	Vomiting
	II	8	6	2	5	2	1	
5 L. J	I	5	7	21	6	2	1	
	II	1	12	1	1	1	1	
6 A M J	I	1	1	22	7	3	2	
	II	1	1	11	4	2	3	
7 B A R.	I	1	1	25	6	2	3	Vomiting
	II	1	14	7	4	3	1	
8 P H	I	2	4	19	2	1	1	
	II	9	13	28	17	4	9	
9 M S	I	10	1	18	22	11	2	
	II	1	14	9	1	1	1	
Mean	I	4 8	10.5	17.8	7 6	3 7	1 6	
±S E.M		1 6	3.5	2 1	2 0	1 0	0.3	
Mean	II	2.9	14 3	11.5	6.8	3.3	2.3	
±S E.M		1 0	3.2	3 0	2 0	0 8	0 8	

After priming with testosterone

are not harmful Furthermore L-dopa is easily administrated There are however subjects who do not respond to L-dopa (6) Such cases should be retested with other GH stimulatory agents such as insulin and/or arginine in order to exclude or establish more definitely the diagnosis of GH insufficiency

In our study peak GH response was seen at times varying between 30 and 90 minutes after L-dopa administration Thus blood sampling later than 90 minutes following L dopa does not seem necessary

The mechanism whereby L-dopa stimulates GH secretion is not fully understood L-dopa is an immediate precursor of dopamine and norepinephrine These two catecholamines are present in high concentrations in the medial basal hypothalamus and median eminence (2 12) where they seem to function as neurotransmittors (1) Several experimental and clinical studies indicate an important function of these catecholamines as modulators of the anterior pituitary hormone secretion acting either in the hypothalamus or at the pituitary level (3 8 19 26) With this background it has been suggested that L-dopa, which effectively crosses the blood brain barrier acts by increasing dopamine and/or norepinephrine levels in the median eminence and hypothalamus thereby influencing the secretion of the GH releasing hormone (4)

The finding that L-dopa induced GH re-

Table 3 *Effect of L-dopa on thyrotrophin follicle stimulating hormone and luteinizing hormone in plasma, cortisol in serum and blood glucose*

Means ±S.E.M. are tabulated

		Minutes					
		0	30	60	90	120	180
TSH, µU/ml	6	5.5±1.4	4.8±0.9	4.8±1.1	4.7±1.0	5.0±1.2	4.8±1.3
FSH, ng/ml	7	1.1±0.2	1.0±0.[illegible]	1.1±0	0.9±0.1	0.9±0	0.9±0.1
LH, ng/ml	7	1.1±0.1	1.1±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
Cortisol, µg/100 ml	10	17.2±2.4	19.6±2.5	22.1±3.1	23.9±3.4	22.6±3.4	20.1±2.1
Glucose, mg/100 ml	9	75 ±3	85 ±6	77 ±6	76 ±5	77 ±4	75 ±3

lease is blocked by phentolamine suggests that the effect is mediated by α-receptors (5 14)

Animal studies indicate that dopamine plays a role for the release of FSH (13) LH (24) and ACTH (15) It is thus of interest that in our studies L-dopa did not influence either the plasma levels of FSH and LH or the serum cortisol Findings similar to ours have been reported in man by Eddy et al (7).

In our studies a single dose of L-dopa had no effect on the plasma levels of TSH However in euthyroid subjects plasma TSH levels are often at the limit of detection and accordingly a fall in plasma TSH would be difficult to measure. This is of importance because Rapoport et al (22) recently reported that the administration of a single dose of L-dopa to hypothyroid patients with high serum levels of TSH resulted in a *marked* reduction of the TSH levels This could indicate that catecholamines can influence the production of TSH Further support for this hypothesis was given in studies by Nilsson et al (19) They found that the TRH induced increase in plasma TSH was significantly although not markedly reduced following pretreatment with the alpha adrenergic blocker phentolamine

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RESTING METABOLIC RATE IN MALNOURISHED BABIES IN RELATION TO TOTAL BODY POTASSIUM

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ABSTRACT Brooke, O. G., Cocks, Th. and March, Y (Tropical Metabolism Research Unit, University of the West Indies, Kingston, Jamaica). Resting metabolic rate in malnourished babies in relation to total body potassium. Acta Paediatr Scand 63:817 1974.—Fasting metabolic rate was investigated in 33 malnourished children and 17 controls of similar age. Total body potassium (TBK) was also measured in 18 of the malnourished children and in all the controls, so that metabolic rates could be compared in relation to a measure of metabolically active tissue. In newly admitted malnourished children specific potassium depletion was corrected orally while they were given a maintenance diet. Resting metabolism correlated better with TBK than with weight, height or surface area in control and recovered children, but metabolic rate per unit TBK declined with increasing body-weight ($r = -0.51$). This negative correlation became insignificant if metabolic rate was expressed in terms of $TBK^{3/4}$. In the malnourished children resting metabolic rate was reduced compared with control and recovered values, when expressed in terms of weight, height or surface area. The results were confirmed by a comparison of metabolic rates per unit $TBK^{3/4}$, which showed a reduction of about 27% in the malnourished children. No significant difference was found between children with marasmus and those with oedematous malnutrition. During rapid growth fasting metabolism was increased. We conclude that oxygen consumption in metabolically active tissues is reduced in all forms of untreated infantile malnutrition.

KEY WORDS: Basal metabolism, malnutrition, total body potassium, body composition

In 1950 Keys and his associates (20) showed that the resting energy consumption of undernourished adults was low not only in terms of body-weight and surface area, but also in terms of metabolically active tissue. The effect of malnutrition on metabolic rate in childhood is much less clear. Although it has often been reinvestigated since Poppi's original observations in 1900 (37), there has been little consistency in the results which have been reported as low, normal or even high. The reasons for this have been discussed by Rutishauser & McCance (38) and by Ashworth (6) and include such factors as lack of agreement about the definition of malnutrition, variation in the use of sedatives and in the children's activity, low environmental temperature, difficulties in finding suitable reference standards, and failure to make measurements in the fasting state or early enough in the course of hospital treatment. The last factor is very important since resting metabolism often rises early during treatment, especially within 4 h of a meal (2, 5, 9, 23, 29).

Animal work has shown that fasting metabolic rate is lowered both in energy deficiency and in protein deficiency (1, 27, 32). These results were based on oxygen consumption

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Table 1 *Clinical details of 53 malnourished Jamaican children (29 male 24 female) (mean and range)*

	On admission	On discharge
Weight (kg)	5 28 (2.45–8 79)	7 86 (3 65–10 66)
Height (cm)	65 2 (49.5–80 5)	68 1 (57 0–83 0)
*Surface area (m^2)**	0 313 (0 188–0.455)	0 397 (0 243–0.500)
Age (months)	12 1 (4–24)	13 9 (7–27)
Hb (g/100 ml)	8 6 (5 2–10 9)	10 7 (8.8–14 7)
Number of children with		
Oedema	23	–
Skin lesions	22	–
Hair changes	20	–

DuBois 1927

measurements expressed in terms of weight or surface area not of active tissue mass but were evidently not consistent with some of the findings in malnourished human infants Ablett & McCance (2) showed that in kwashiorkor (protein deficiency disease) the fasting metabolic rate was low before treatment when expressed in terms of body weight[?] and rose during recovery We have attempted to confirm these results using a measure of active tissue mass as the unit of comparison and we have carried out similar studies on children with marasmus (deficiency of energy and protein) and on normal controls in the same age range We have used total body potassium (TBK) as a measure of metabolically active tissues since potassium is present in high concentration in these tissues (19 40) and it has been shown that there is a direct relationship between TBK and fat free dehydrated protoplasmic mass (3) and between TBK and resting metabolic rate (3 25) Preliminary results of these studies have already been published (10)

MATERIAL AND METHODS

Subjects Seventy children were studied. Fifty-three of these were admitted to the Tropical Metabolism Research Unit because of severe primary malnutrition, and there were 17 controls. Some facts about the malnourished children are given in Table 1 On admission they were on average 57% of expected weight for age and 72% of expected weight for height using the fiftieth centile of the Boston standards (33) When they were discharged they were 76 and 98% of these parameters respectively On the basis of the classification of the Wellcome Trust Working Party on Malnutrition (24) 30 were marasmic or underweight 14 had marasmic-kwashiorkor and 9 had kwashiorkor The children were not selected in any way except for the exclusion from the series of those who were persistently febrile during their first week of treatment. All the children were studied at least twice—on admission and before discharge—and in some cases more often Metabolic rate (MR) was measured in every child and TBK in 18 consecutively admitted malnourished children and the 17 controls whose details are given in Table 2. Initial MR determination was done within 24 h of admission in all children who had TBK measurement, and within 72 h in the remainder During this period the children were on a maintenance diet providing 419 kJ and 0 7 g protein per kg body-weight daily (in line with current international nomenclature all energy values are expressed in joules[1]).

Fourteen of the 17 controls came from a Home of Safety for abandoned children for which medical care was provided by physicians from the Unit. They were all well nourished The opportunity to study their metabolic rates was provided by a concurrent study of the absorption of iron from staple cereals (Ann Ashworth to be published) which required the children to be brought fasting to the Unit and involved whole body counting, for which sedation is generally given in small children The only additional procedure was the measurement of O_2 consumption. The other controls were patients in the Paediatric Unit of the University Hospital who had recovered from non-nutritional disorders and who were also well-nourished. The controls were on average 97% of their expected weight for age and 94% of their expected weight for height.

Metabolic rate measurements All determinations were made in the same way For administrative reasons the 14 controls from the Home of Safety were studied in the late morning and the other children in the early afternoon No measurements were made less than 4.5 h after the last feed All the children were studied in a metabolic chamber (8) in which the environmental temperature was between 30 and 33°C and the relative humidity between 40 and 50% Sedation was used because in our experience and that of others (13 18) the results are thus made more consistent We gave paraldehyde in a standard dose (0.5 ml/kg body-weight by enema) since it is safe and reliable (26) and since we were familiar with its use in malnourished children The children were not placed in the chamber until they were asleep and 30 min were allowed for settling down and equilibration before measurements began.

Oxygen consumption and CO_2 evolution were measured with a Noyons diaferometer (Kipp & Zonen, Delft, Holland) calibrated by burning alcohol (31)—repeated

[1] 1 kcal=4 184 kJ

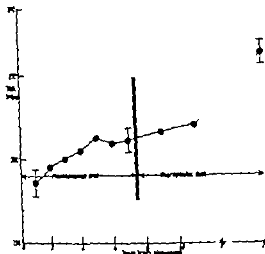


Fig. 1. Changes in mean total body potassium (TBK) in 18 malnourished children during their first 25 days of hospital treatment. Bars indicate standard errors.

determinations at the same burning rate varying by less than 5%. At least 2 determinations were made during each study, more if the first two varied widely, and the mean was used to calculate the metabolic rate. Each determination lasted at least 15 min.

Total body potassium. Measurements of TBK were made on a Packard 4π liquid scintillation whole body counter (15). Counts were made to an accuracy of ±6% and the results were corrected for self-absorption.

TBK in malnourished children is invariably low, not only because of the reduction in bulk of potassium-containing tissues but also because of specific potassium depletion resulting from diarrhoea and other factors (4). Thus the usual value does not reflect the amount of metabolically active tissue in the body until specific depletion is corrected. To get round this difficulty the malnourished children were kept on a maintenance diet which provided 419 kJ and 0.7 g protein/kg body-weight daily during their first week in hospital. This halted further loss of weight while water and electrolyte disturbances were corrected but did not allow growth. Oral potassium supplements (6 mEq/kg body-weight daily) were given during this week and TBK was measured daily. Fig. 1 shows the change in mean TBK in 18 children during the first 25 days of treatment. The values reached a plateau by the 5th day and initial MRs were expressed in terms of the mean TBK on the 6th and 7th days. The difference in TBK on these days was never greater than 8 mEq. In the case of the recovered and control children, and in those who were studied during rapid growth, MR was expressed in terms of TBK measured on the same day.

Ethical considerations. Parental consent for the studies was obtained, and in the case of the controls the consent of the Matron of the Home of Safety, who was their legal guardian. The use of a maintenance diet during the first week of treatment is in line with generally recognised principles of management—that initial refeeding should be gradual and that a therapeutic feeding regimen should only be introduced after the correction of fluid and electrolyte disturbances.

RESULTS

Controls. Individual metabolic rate and TBK results are given in Table 2. The mean daily heat production of the controls was 103% (S.E. ±2.4%) of Talbot's standards (39). Mean TBK was 46.6 mEq/kg body weight (S.E. ±0.8) which agrees well with the expected value of 46.0 mEq/kg for a Jamaican child of 1 year (16). There is also close agreement with values found in North American infants (35). The following correlations were found between heat production (y) in kJ/day and several independent variables (x) in the 17 controls.

x (independent variable)	r	t	P	Regression equation
1. Weight (kg)	+0.64	3	<0.01	$y = 123.4x + 1020$
2. Height (cm)	+0.36	1.5	>0.05	$y = 31.6x - 169.6$
3. Surface area (m^2)	+0.64	3.2	<0.01	$y = 4790x - 10.3$
4. Weight$^{3/4}$ (kg)	+0.64	3.2	<0.01	$y = 778.4x - 684$
5. Total body K (mEq)	+0.87	6.9	<0.001	$y = 3.26x + 741$

Metabolic rate correlated best with TBK and the correlation with height was poor in this small series of normal children. Correlations between metabolic rate and the anthropometric data were improved by considering the recovered children as well. In this case the correlation with weight increased to $r = +0.80$, with height to $r = +0.69$ ($t = 5.2$, $p < 0.001$), with surface area to $r = +0.72$, and with weight to $r = +0.78$. The correlation with TBK was degraded slightly by including the recovered children ($r = +0.86$) but it remained the best of the measures we used and is shown in Fig. 2. For these comparisons only the 18 recovered children in whom TBK was measured were included, so the total number of subjects was 35 in each case. Analysis of variance showed no significant difference between the slopes for the recovered and control children.

Expression of metabolic rate. We have

Table 2 *Clinical details metabolic rate and total body potassium in 18 malnourished children and 17 controls*

A = on admission R = recovered In children with oedema lowest weight is given

Case	Sex	Oedema	Age (months) A	Age (months) R	Weight (kg) A	Weight (kg) R	Height (cm) A	Height (cm) R	MR (kJ/d) A	MR (kJ/d) R	TBK (mEq) A	TBK (mEq) R
Malnourished												
1	F	0	6	8	3.97	6.35	58.5	6[illegible].5	980	1 758	221	318
2	M	0	7	9	4.32	7.07	61.0	66.75	1 082	1 909	224	337
3	F	0	8	10	5.19	7.44	65.5	66.0	954	1 813	196	301
4	M	0	10	13	6.43	8.79	68.25	71.0	1 35[illegible]	1 825	300	381
5	M	0	11	13	5.13	9.52	69.0	73.5	1 063	2 286	252	386
6	M	0	13	15	5.97	8.58	69.5	71.0	1 076	2 085	284	356
7	M	0	15	17	6.99	9.07	72.0	75.5	1 256	2 190	311	457
8	M	0	16	17	5.76	8.56	68.5	71.0	1 090	2 303	260	433
9	F	0	16	19	7.33	10.21	75.5	76.0	1 696	2 378	36[illegible]	477
10	F	0	19	21	3.68	6.74	61.0	65.5	946	1 821	201	263
11	F	+	6	7	3.36	5.19	55.0	58.5	846	1 298	167	266
12	M	+	8	10	4.42	5.68	61.0	63.5	925	1 696	179	256
13	M	+	12	14	5.83	8.43	65.5	70.0	1 097	2 279	220	455
14	F	+	12	14	6.35	8.75	68.0	71.0	1 189	2 144	283	378
15	F	+	13	15	5.19	8.13	63.25	66.5	1 076	1 951	242	351
16	F	+	14	15	6.96	9.66	72.0	75.75	1 114	2 504	191	461
17	M	+	14	16	8.25	10.58	73.5	77.25	1 176	2 738	314	503
18	M	+	22	23	5.84	8.72	71.0	72.5	1 155	2 169	274	371
Controls												
1	M		8		7.72		74.0		1 700		320	
2	F		8		7.31		68.0		2 014		370	
3	M		9		7.33		70.5		2 064		355	
4	M		9		7.89		67.0		1 871		356	
5	M		10		8.77		69.75		1 930		399	
6	M		11		9.55		72.5		386		436	
7	M		11		9.28		71.0		2 043		403	
8	M		11		8.45		75.0		2 164		414	
9	F		1		9.80		75.0		2 395		503	
10	F		12		8.93		73.0		2 118		442	
11	M		12		9.57		71.5		2 382		461	
12	M		13		9.66		75.0		2 349		462	
13	F		14		9.58		75.0		2 428		494	
14	F		15		10.47		73.5		060		461	
15	M		15		11.39		73.5		2 315		493	
16	M		17		10.84		78.0		2 282		499	
17	F		19		8.63		76.5		1 876		360	

compared the metabolic rates of the malnourished recovered and control children in terms of the usual anthropometric data (weight weight$^{3/4}$ surface area and height) and also in terms of TBK However the control children though of similar age were larger than the malnourished children (their mean weight was 73% greater) so comparison of metabolic rates in terms of weight or surface area will not only be unsatisfactory because of changes in body composition but also because of the systematic changes which are known to occur with increasing body weight (22) For example in our 53 recovered children we found the following correlations

MR (kJ/kg/day) vs weight, $r = -0.42$ $t = 3.4$ $p < 0.01$
MR (kJ/m²/day) vs weight, $r = +0.41$ $t = 3.3$ $p < 0.01$
MR (kJ/100 cm/day) vs weight $r = +0.63$ $t = 6.0$ $p < 0.00$
MR (kJ/kg$^{3/4}$/day) vs. weight, $r = +0.30$ $t = 2.2$ $p < 0.05$

Thus the error in comparing children of appreciably different weight will be greatest when MRs are expressed per unit length and least when expressed per unit weight$^{3/4}$

Table 2 *Clinical details metabolic rate and total body potassium in 18 malnourished children and 17 controls*

A = on admission R = recovered. In children with oedema lowest weight is given

Case	Sex	Oedema	Age (months) A	Age (months) R	Weight (kg) A	Weight (kg) R	Height (cm) A	Height (cm) R	MR (kJ/d) A	MR (kJ/d) R	TBK (mEq) A	TBK (mEq) R
Malnourished												
1	F	0	6	8	3 97	6.35	58 5	62.5	980	1 758	221	318
2	M	0	7	9	4 32	7 07	61 0	66 75	1 082	1 909	224	337
3	F	0	8	10	5 19	7 44	65.5	66 0	954	1 813	196	301
4	M	0	10	13	6.43	8 79	68.5	71 0	1 352	1 825	300	381
5	M	0	11	13	5 13	9 52	69 0	73.5	1 063	2 286	252	386
6	M	0	13	15	5 97	8.58	69.5	71 0	1 026	2 085	284	356
7	M	0	15	17	6.99	9 07	72 0	75.5	1 256	190	311	457
8	M	0	16	17	5 76	8.56	68.5	71 0	1 080	2 303	260	433
9	F	0	16	19	7 33	10 21	75 5	76.0	1 696	2 378	362	477
10	F	0	19	21	3 68	6 74	61 0	65.5	946	1 821	201	263
11	F	+	6	7	3 36	5 19	55 0	58.5	846	1 298	167	266
12	M	+	8	10	4 42	5 68	61 0	63.5	925	1 696	179	256
13	M	+	12	14	5 83	8 43	65.5	70 0	1 097	2 279	[illegible]20	455
14	F	+	12	14	6 35	8 75	68 0	71 0	1 189	2 144	283	378
15	F	+	13	15	5 19	8 13	63 25	66.5	1 076	1 951	242	351
16	F	+	14	15	6 96	9 66	72 0	75 75	1 114	2 504	191	461
17	M	+	14	16	8 25	10.58	73.5	77.25	1 176	2 738	314	503
18	M	+	22	23	5 84	8 72	71 0	72.5	1 155	2 169	274	371
Controls												
1	M		8		7 72		74 0		1 700		370	
2	F		8		7 31		68 0		014		370	
3	M		9		7 33		70.5		2 064		355	
4	M		9		7 89		67 0		1 871		356	
5	M		10		8 77		69 75		1 930		399	
6	M		11		9 55		72.5		2 386		436	
7	M		11		9 28		71 0		2 043		403	
8	M		11		8 45		75 0		2 164		414	
9	F		12		9 80		75 0		2 395		503	
10	F		12		8 93		73 0		2 118		442	
11	M		12		9 57		71.5		2 382		461	
12	M		13		9 66		75 0		2 349		462	
13	F		14		9 58		75 0		2 4[illegible]8		494	
14	F		15		10 47		73.5		060		461	
15	M		15		11 39		73 5		315		493	
16	M		17		10.84		78 0		2 282		499	
17	F		19		8 63		76 5		1 876		360	

compared the metabolic rates of the malnourished recovered and control children in terms of the usual anthropometric data (weight weight$^{3/4}$ surface area and height) and also in terms of TBK However the control children though of similar age were larger than the malnourished children (their mean weight was 73% greater) so comparison of metabolic rates in terms of weight or surface area will not only be unsatisfactory because of changes in body composition but also because of the systematic changes which are known to occur with increasing body weight (22) For example in our 53 recovered children we found the following correlations

MR (kJ/kg/day) vs weight $r=-0.42$ $t=3.4$ $p<0.01$
MR (kJ/m²/day) vs weight $r=+0.41$ $t=3.3$ $p<0.01$
MR (kJ/100 cm/day) vs weight $r=+0.63$ $t=6.0$, $p<0.001$
MR (kJ/kg$^{3/4}$/day) vs. weight $r=+0.30$, $t=2.2$, $p<0.05$

Thus the error in comparing children of appreciably different weight will be greatest when MRs are expressed per unit length and least when expressed per unit weight$^{3/4}$.

Table 4 *Metabolic rate (kJ/mEq TBK⁻¹/day) in marasmus marasmic kwashiorkor and kwashiorkor on admission to hospital*

Marasmus		Marasmic kwashiorkor		Kwashiorkor	
Case	MR	Case	MR	Case	MR
1	17 04	11	18 21	14	16 70
2	18 67	12	18 88	16	21 85
3	18 71	13	18 61	17	15 78
4	18 76	15	17 50		
5	16.83	18	17 04		
6	14 86				
7	16.96				
8	16 70				
9	20 18				
10	17 75				
Mean	17 60		18 04		18 13

phase of accelerated growth when their average weight gain was about 10 g/kg body weight daily During this period their fasting MR was elevated above the recovered values and the difference was statistically significant using the *t*-test applied to paired comparison ($t=2\ 75$ $p<0\ 01$)

Table 4 shows a comparison of the MRs of marasmic children with those of the children with kwashiorkor and marasmic kwashiorkor Expressed in terms of TBK the average MR was lower in marasmus than in the oedematous forms of malnutrition but the difference was not significant The number of children with kwashiorkor was however very small

DISCUSSION

The difficulty in finding a suitable reference standard for the comparison of metabolic rates was recognised long ago and Benedict and Talbot (7) discussed the problem at length in 1914 Loss of fat in marasmus results in a relative increase in the metabolically active tissues and artificially raises metabolic rate when it is expressed in terms of body weight or surface area Similarly the increased inert weight in oedematous malnutrition may lead to an apparent reduction on metabolic rate Benedict & Talbot (7) found that heat production in their marasmic cases did not follow Rubner s surface law and wrote (1914) —we find ourselves thoroughly convinced that the metabolism is determined not by the body surface but by the active mass of protoplasmic tissue In spite of this there has been only one attempt to express the metabolic rates of malnourished children in such terms (using body solid mass derived from measurements of total body water) and no comparative measurements were made on controls (29)

Another drawback to the use of conventional reference standards when comparing metabolic rates in malnourished and normal children is that if children of like weight or height are selected they may not be of comparable age whereas children of similar age but different nutritional state will vary widely in physical dimensions In the latter errors will arise because of the changes in MR per unit weight or surface area with increasing body weight described by Kleiber (22) These changes result from alterations in the ratio of metabolically active tissue to external physical dimensions during growth and cause an apparent increase in MR/m² with increasing body weight and an apparent decrease in MR/kg Similar errors occur when MR is expressed in terms of height as we have shown in this paper Their effect is considerable For instance recalculation of the metabolic rates of 13 malnourished children (hypothrepsiques du 2 degré) studied by Garot (14) shows that on average they were +14% −8 5% −16% and −39% of a normal standard when expressed in terms of body weight weight^¾ surface area and height respectively It was because of this kind of uncertainty that Kleiber (21) recommended the use of weight^¾ or metabolic body size in the comparison of metabolic rates since the correlation between MR/kg⁻¹ and body weight is usually insignificant over a wide range of different sized mammalian species

Our more limited experience with total

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tion is evidently greater than the difference in the total oxygen consumption when compared in terms of potassium content Table 5 also shows why the correlation between MR and TBK though demonstrably better than similar correlations with anthropometric data falls short of that which could be attained if one were able to identify the perfect marker of metabolically active tissues Potassium cannot fulfill this latter rôle since about half of the TBK is found in the muscles whereas only about 10% of the total metabolism takes place in them Furthermore although the brain liver heart and kidneys account for about two thirds of the total metabolism these organs do not contain more than one third of the TBK

The reason for the reduction in metabolic rate in undernutrition is not known Although it has often been claimed that it is due to hypothyroidism there is no evidence that undernourished animals behave as hypothyroid animals and a recent article reports values for serum thyroxine and free thyroxine index at the upper limits of the normal range in untreated marasmic children (36) With present techniques it is impossible to determine whether the decrease in total oxygen consumption reflects a decreased metabolism in every cell or whether it represents a reduction in size or energy expenditure in one or more of the principal oxygen-consuming organs for example the brain The increase in fasting metabolism to supranormal levels during recovery has been noted before (29) and in conjunction with the large rise in post prandial energy expenditure which occurs during rapid growth may be a reflection of the energy cost of new tissue synthesis (9) These fascinating changes deserve further study

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CLINICAL ASPECTS OF NEONATAL HYPOGLYCAEMIA

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ABSTRACT Fluge G (Department of Paediatrics, University of Bergen Bergen Norway) Clinical aspects of neonatal hypoglycaemia Acta Paediatr Scand 63 826, 1974.—Fifty cases of neonatal hypoglycaemia were detected by routine blood glucose determination in 323 low birth weight infants during a three-year period (15.4%) and in addition, hypoglycaemia was diagnosed in 17 full-term infants. The patients were divided in three groups according to clinical findings, with special reference to age at diagnosis, pretreatment blood glucose values and duration of hypoglycaemia In *asymptomatic* hypoglycaemia the diagnosis was made during the first few hours after birth and the mean pretreatment blood glucose value was 14 mg/100 ml Except for one patient the hypoglycaemia was of short duration *Symptomatic transient* hypoglycaemia was characterized by a delay in onset of symptoms until the second and third day after birth low pretreatment blood glucose level and hypoglycaemia of long duration. Hypoglycaemia associated with other neonatal disorders classified as *secondary* hypoglycaemia usually was noted during the first few hours of life and tended to be of short duration. Frequency of hypoglycaemia in small for gestational age infants was markedly higher when toxaemia of pregnancy was noted compared with infants born to non-toxaemic mothers.

KEY WORD: Neonatal hypoglycaemia

Hypoglycaemia is rather common in newborn infants The association between neonatal hypoglycaemia and abnormal neurological manifestations was first emphasized by Cornblath et al in 1959 (4) The symptoms attributed to hypoglycaemia are however complex and there are no pathognomonic signs The diagnosis is dependent on the finding of a low blood glucose level although the clinical significance of this is often difficult to assess (7)

In the present investigation hypoglycaemic newborn infants have been classified in three different groups according to clinical findings with special reference to age at time of diagnosis initial blood glucose values and duration of hypoglycaemia

MATERIAL AND METHODS

During the three year period 1967–1969 blood glucose was recorded in 323 consecutive patients with a birth weight of 2500 g or less admitted to the Department of Paediatrics University of Bergen Blood glucose level was estimated within 24 hours of admission and followed during the next few days. *In full-term infants* admitted during the same period blood glucose determinations were made whenever symptoms suggestive of hypoglycaemia were observed Infants of diabetic mothers were excluded

The infants were fed within 4–6 hours of birth with human milk or cow's milk formula extra 5% glucose solution being added. The calorie intake increased from approximately 11 5–14 kcal/kg body weight on the first day to 96–130 kcal/kg by the end of the second week.

Hypoglycaemia was defined as a blood glucose level below 20 mg/100 ml in low birth weight infants and below 30 mg/100 ml in full-term infants during the first 72 hours of life and below 40 mg/100 ml after this age

The hypoglycaemic infants were divided into three

Table I *Neonatal hypoglycaemia in relation to toxaemia of pregnancy*

Number low birth weight infants		Percentiles birth weight/gest. age (Lubchenco et al.)		Number of infants with neonatal hypoglycaemia: Low birth weight infants	Full-term infants	Total number
Singletons, toxaemia of pregnancy	40	Above 10th percentile	16 (40%)	-/16	5	7
		Below 10th percentile	4 (60%)	9/?4	0	9
Twins/triplets	61	Toxaemia: 5		5/61 (tox.)		7
Singletons, non-toxaemic mothers	222	Above 10th percentile	178 (80%)	26/178	9	35
		Below 10th percentile	44 (20%)	8/44	1	9
	323			50/323	17	67

groups according to clinical assessment of presenting symptoms

Group A. *Asymptomatic hypoglycaemia.* No symptoms attributable to hypoglycaemia.

Group B. *Symptomatic transient hypoglycaemia.* Symptomatic hypoglycaemia responding to glucose infusion. No other neonatal disorders apparent.

Group C. *Secondary hypoglycaemia.* Hypoglycaemia in patients with other neonatal disorders and without obvious response to glucose infusions.

Hypoglycaemic infants were treated by intravenous administration of 50% glucose solution 1.2 ml/kg body weight, followed by continuous infusion of 20–10% solution sufficient to maintain blood glucose at a level of 40–70 mg/100 ml. If glucose levels were not satisfactory on this treatment 4 units ACTH every 12 hours or 5 mg/kg body weight hydrocortisone per day was given by intramuscular injection. When blood glucose level had stabilized, oral feeding with human milk or cow's milk formula was gradually introduced. In some patients oral feeding with 10% glucose solution alone was sufficient to alleviate the hypoglycaemia.

Blood samples for glucose determination were drawn from heel punctures after rubbing, immediately deproteinized with trichloracetic acid and analyzed by the ortho-toluidin method. For statistical analyses the Student *t*-test was applied.

RESULTS

Among 323 low birth weight infants hypoglycaemia was detected in 50 patients (15.4%). In addition 17 patients with birth weight above 2500 g were hypoglycaemic. The material thus consists of 67 patients. 34 were males and 33 f...

Birth history and occurrence of toxaemia of pregnancy

A history of toxaemia of pregnancy was noted in 40 low birth weight singletons (12%). Eleven of these (27.5%) became hypoglycaemic and 9 of them were small for gestational age (SGA) with a birth weight below the 10th percentile according to Lubchenco et al. (11). Among 222 low birth weight singletons born to non-toxaemic mothers 34 (15.4%) showed hypoglycaemia and 8 of them (23.5%) were SGA babies. Five out of 61 twins (8%) became hypoglycaemic and toxaemia was present in 2 of the mothers. The frequency of SGA infants among the normoglycaemic singletons was 60% when the mother had toxaemia of pregnancy and 20% when the mother was non-toxaemic (Table 1).

Data from birth histories are presented in Table 2. The occurrence of SGA babies was equally represented in the three groups. In groups A and B all but one of the SGA infants were born to toxaemic mothers while in group C this was only so in 4 out of 11 cases. Twenty-two infants in group C had signs of light to moderate asphyxia at birth while 7 patients were severely asphyxiated. Four of them died during the first 24 hours after birth.

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Hypoglycaemia was defined as a blood glucose level below 20 mg/100 ml in low birth weight infants and below 30 mg/100 ml in full-term infants during the first 72 hours of life and below 40 mg/100 ml after this age

The hypoglycaemic infants were divided into three

Table 2 *Birth history in 67 patients with neonatal hypoglycaemia*

Category of hypoglycaemia	No of cases	Toxaemia	Surgical intervention*	Asphyxia	Birth weight below 10th perc	Maternal age years (mean and range)	Gest age weeks (mean ±1 S D)
Asymptomatic (group A)	13	3	2	0	4	27 2 (20–39)	33 9±4.2
Symptomatic transient (group B)	11	4	4	0	3	25 9 (18–32)	37 9±2 9
Secondary (group C)	43	11	1	29	11	27 0 (17–45)	35 0±4 5

* Caesarian section forceps delivery and other obstetrical manoeuvres

Age at time of diagnosis

In most instances the diagnosis of asymptomatic hypoglycaemia was made during the first few hours after birth while the infants of group B usually became symptomatic during the second day The difference is highly significant Age at diagnosis in secondary hypoglycaemia was also significantly lower than in the symptomatic group (Table 3)

When the infants in groups B and C were divided into a convulsive and a non-convulsive group it appeared that the diagnosis was made at a younger age in the non-convulsive infants (Table 4) These differences however were not significant

Pretreatment blood glucose values

Initial blood glucose values are presented in Table 3 In asymptomatic hypoglycaemia the mean value was significantly higher than in symptomatic hypoglycaemia, while in secondary hypoglycaemia the mean pretreatment value was in between that found in groups A and B Those patients who died during the neonatal period had a mean pretreatment blood glucose value of 8 mg/100 ml which was significantly lower than that found in group A ($p<0.005$) but corresponded well with the mean levels found in groups B and C Subgrouping the infants in groups B and C into a convulsive and a non-convulsive group disclosed lower pretreatment blood glucose levels in the convulsion groups than in the non-convulsion groups (Table 4)

Duration of hypoglycaemia

Duration of hypoglycaemia from occurrence of symptoms until blood glucose had stabilized at a normal level was usually more than 12 hours in group B (10 out of 11 patients)

Table 3 *Clinical data in different categories of neonatal hypoglycaemia*

Category of hypoglycaemia	No of cases	Age at diagnosis hours mean ±1 S D (range)	Pretreatment blood glucose mg/100 ml mean ±1 S D (range)	Duration of hypoglycaemia more than 1 hours	Convulsions
Asymptomatic (group A)	13	4 5±7 9 (1–29)	14±5 (5–19)	1	0
		$p<0.001$	$p<0.01$		
Symptomatic, transient (group B)	11	38 0±31 7 (2–77)	8±4 (2–15)	10	7
		$p<0.001$	$p>0.1$		
Secondary (group C)	43	7.5±15 7 (1–68)	11±6 (0–19)	11	9

respectively) These observations might suggest that symptomatic transient hypoglycaemia detected during the second and third day may be preceded by low glucose levels at an earlier age Similar observations were noted by Raivio (16) and Koivisto et al (10)

In secondary hypoglycaemia (group C in the present material) age at diagnosis is significantly lower than in the symptomatic group The hypoglycaemia is usually of short duration and is easy to correct with glucose it without concomitant change in the clinical condition. The deleterious effect of low glucose levels in these patients is additional to other factors (brain injury respiratory distress etc) and is probably of minor clinical importance regarding presenting symptoms One must bear in mind however that hypoglycaemia per se causes brain damage (1 2) and we do not know at present to what extent a delay in the detection of hypoglycaemia may aggravate this harmful effect on the central nervous system

Convulsions have been claimed to be a serious symptom in neonatal hypoglycaemia indicating a bad prognosis (10) In the present series convulsions seemed to be related to diagnosis at a later age low pretreatment blood glucose values and probably also a longstanding hypoglycaemia The method of glucose determination is inaccurate at very low levels (less than 10 mg/100 ml) thus making it difficult to assess the significance of very low pretreatment blood glucose levels However it seems reasonable to assume that the above mentioned conditions indicate severe disturbances of the central nervous system leading to both convulsions and permanent damage

It has been questioned whether asymptomatic hypoglycaemia is of clinical significance (9 10) Most investigators agree that asymptomatic hypoglycaemia represents a potential hazard to the central nervous system (3) although signs of permanent injury are less frequently noted at follow-up examination (9 10) compared with that found in symptomatic hypoglycaemia. Gentz et al (6) found a normal A value during intravenous glucose tolerance tests in infants with asymptomatic hypoglycaemia, which indicated normal peripheral utilization of glucose In the present material pretreatment blood glucose level was significantly higher in asymptomatic hypoglycaemia than in symptomatic hypoglycaemia and except for one patient the duration of hypoglycaemia was short. The diagnosis was usually made during the first few hours after birth It is therefore reasonable to assume that the deleterious effect of hypoglycaemia in these patients would be much less than in symptomatic transient hypoglycaemia.

The frequency of hypoglycaemia among low birth weight infants in this material was 15 4% which is a relatively high incidence However it corresponds well with the findings of Griffiths (7) who reported an incidence of 14.8% among infants admitted to a special care unit. Our material is also selected as most premature babies weighing more than 2000 g were observed in the Maternity Unit and were not transferred to the Children s Hospital The feeding schedule for prematures in our hospital during the six ties did not offer enough calories according to the standards of to-day Although feeding was started at an early age (4–6 hours) the low caloric intake may account in part for the high incidence of hypoglycaemia.

The relation between SGA babies associated with toxaemia of pregnancy and hypoglycaemia has been repeatedly demonstrated by several investigators (6 12 13 14 15) These observations are confirmed in the present material since both the relative number of SGA infants and the frequency of hypoglycaemia was markedly higher in babies born to toxaemic mothers compared with those born to non-toxaemic mothers Among infants of the latter category the relative numbers of SGA babies were however about the same in both hypoglycaemic and normo-

Table 6 *Autopsy findings in nineteen patients dying with neonatal hypoglycaemia*

	Category of hypoglycaemia		
	Asymptomatic	Symptomatic transient	Secondary
Number of patients	1	1	17*
Pulmonary atelectasis and hyaline membranes	1	0	14
Cerebral oedema	0	0	4
Intracranial haemorrhage	0	0	1
Ruptured meninges	0	0	[illegible]

In some patients more than one pathological finding was noted

initially had asymptomatic hypoglycaemia She was immature with a birth weight of 770 grams Terminally she had attacks of cyanosis and apnoea and was normo-gly caemic She died on the 5th day Autopsy revealed pulmonary atelectasis and hyaline membranes adrenal haemorrhages and hy perplasia of the islets of Langerhans

*One male infant with symptomatic hypo-*glycaemia showed symptoms of central ner vous system irritation at 40 hours of age Hypoglycaemia had probably existed for 20 hours before admission By the time glucose infusions were given he was already dying and therapy was in vain At autopsy no ab-normal findings were noted except for some haemorrhage in the lungs which was inter preted as a terminal phenomenon Unfor tunately microscopic examination of the brain was not undertaken

In secondary hypoglycaemia pulmonary atelectasis and hyaline membranes were most often noted Four patients had intracranial complications and seven had minor congenital malformations

DISCUSSION

Clinical assessment of presenting symptoms in neonatal hypoglycaemia is difficult since similar symptoms can be caused by a variety of conditions such as perinatal anoxia intra cranial hemorrhage meningitis or hypocal caemia These symptoms include attacks of cyanosis and apnoea, muscular hypotonia apathy weak cry refusal to take food and convulsions In a study of 1000 consecutive newborns admitted to a special care unit Griffiths (7) studied the occurrence of such symptoms in relation to neonatal hypo-glycaemia He concluded that in the majority of infants symptoms were seemingly un related to the presence of hypoglycaemia. Furthermore he could not demonstrate any increase in severity of symptoms in hypo-glycaemic infants compared to those with normal glucose levels However when symp-toms coincide with the finding of low glucose values and favourable response to glucose infusions is noted it seems reasonable to relate symptoms to the hypoglycaemia (5 13 15) The diagnosis of symptomatic tran sient neonatal hypoglycaemia should be re stricted to patients fulfilling these criteria. In the present material several patients of this category were admitted from other hos-pitals and nurseries during the second and third day after birth because of convulsions Nothing is known of the blood glucose levels in these patients before admission nor of the accuracy of the clinical observation as to the presence of minor hypoglycaemic symp-toms Only four group B infants were fol lowed from birth on They were all hypo-glycaemic during the first few hours after birth three of them being symptomatic with rapid clinical response to glucose infusions In these infants there was a tendency to re current fall in blood glucose level during the second and third day (8 9 15 26 ml/100 ml

respectively). These observations might suggest that symptomatic transient hypoglycaemia detected during the second and third day may be preceded by low glucose levels at an earlier age. Similar observations were noted by Raivio (16) and Koivisto et al (10).

In secondary hypoglycaemia (group C in the present material) age at diagnosis is significantly lower than in the symptomatic group. The hypoglycaemia is usually of short duration and is easy to correct with glucose but without concomitant change in the clinical condition. The deleterious effect of low glucose levels in these patients is additional to other factors (brain injury, respiratory distress etc.) and is probably of minor clinical importance regarding presenting symptoms. One must bear in mind, however, that hypoglycaemia per se causes brain damage (1, 2) and we do not know at present to what extent a delay in the detection of hypoglycaemia may aggravate this harmful effect on the central nervous system.

Convulsions have been claimed to be a serious symptom in neonatal hypoglycaemia indicating a bad prognosis (10). In the present series convulsions seemed to be related to diagnosis at a later age, low pretreatment blood glucose values and probably also a longstanding hypoglycaemia. The method of glucose determination is inaccurate at very low levels (less than 10 mg/100 ml) thus making it difficult to assess the significance of very low pretreatment blood glucose levels. However, it seems reasonable to assume that the above mentioned conditions indicate severe disturbances of the central nervous system leading to both convulsions and permanent damage.

It has been questioned whether asymptomatic hypoglycaemia is of clinical significance (9, 10). Most investigators agree that asymptomatic hypoglycaemia represents a potential hazard to the central nervous system (3), although signs of permanent injury are less frequently noted at follow-up examination (9, 10) compared with that found in symptomatic hypoglycaemia. Gentz et al (6) found a normal A value during intravenous glucose tolerance tests in infants with asymptomatic hypoglycaemia, which indicated normal peripheral utilization of glucose. In the present material pretreatment blood glucose level was significantly higher in asymptomatic hypoglycaemia than in symptomatic hypoglycaemia and, except for one patient, the duration of hypoglycaemia was short. The diagnosis was usually made during the first few hours after birth. It is therefore reasonable to assume that the deleterious effect of hypoglycaemia in these patients would be much less than in symptomatic transient hypoglycaemia.

The frequency of hypoglycaemia among low birth weight infants in this material was 15.4%, which is a relatively high incidence. However, it corresponds well with the findings of Griffiths (7) who reported an incidence of 14.8% among infants admitted to a special care unit. Our material is also selected, as most premature babies weighing more than 2000 g were observed in the Maternity Unit and were not transferred to the Children's Hospital. The feeding schedule for prematures in our hospital during the sixties did not offer enough calories according to the standards of to-day. Although feeding was started at an early age (4–6 hours) the low caloric intake may account in part for the high incidence of hypoglycaemia.

The relation between SGA babies associated with toxaemia of pregnancy and hypoglycaemia has been repeatedly demonstrated by several investigators (6, 12, 13, 14, 15). These observations are confirmed in the present material, since both the relative number of SGA infants and the frequency of hypoglycaemia was markedly higher in babies born to toxaemic mothers compared with those born to non-toxaemic mothers. Among infants of the latter category the relative numbers of SGA babies were, however, about the same in both hypoglycaemic and normo-

glycaemic infants indicating that SGA babies born to non-toxaemic mothers do not have the same tendency to develop hypoglycaemia as when toxaemia is present

Routine blood glucose screening in all new born infants at risk should always be carried out for several days Even full-term newborns with a normal birth history may develop hypoglycaemia and close observation in neonates of all categories is therefore necessary Even minor symptoms should prompt a blood glucose estimation in order to detect hypoglycaemia as early as possible and before serious symptoms occur

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IMMUNOELECTROPHORETIC DETERMINATION OF SERUM GLOBULINS IN NEWBORN INFANTS OF DIABETIC MOTHERS

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ABSTRACT Davidsen, Otto (Diabetes Centre, Royal Maternity Department B, Rigshospitalet and Department of Clinical Chemistry Sundby Hospital, Copenhagen, Denmark) Immunoelectrophoretic Determination of Serum Globulins in Newborn Infants of Diabetic Mothers. Acta Paediatr Scand, 63: 833, 1974.—Serum globulins were investigated by means of crossed immunoelectrophoresis in the cord blood from 40 infants of diabetic mothers and 93 infants with non-diabetic mothers. In infants of non-diabetic mothers the concentration of most globulins was lower than in adults and positively correlated to the gestational age of the infant. For α_2-HS-glycoprotein and an unidentified α_2-globulin, however negative correlation to the gestational age was observed. Infants of diabetic mothers had higher concentration of transferrin and lower concentrations of α_2-macroglobulin and α_1-lipoprotein as compared with infants of the reference group. In the diabetes group the globulin concentrations were correlated neither to the gestational age nor to their increased birth weight, but the ratio α_2-macroglobulin/α_2-HS-glycoprotein, which was expected to be independent of variations in the degree of hydremia of the infants, was significantly correlated to the gestational age As judged from this parameter infants of diabetic mothers are comparable to infants of non-diabetic mothers of about 4 weeks lower gestational age

KEY WORDS: Newborn infants, infants of diabetic mothers, serum globulins, α_2-HS-glycoprotein, transferrin, α_2-macroglobulin α_1-lipoprotein

In newborn infants of diabetic mothers the birth weight is higher than would be expected from the gestational age which generally is about 3 to 4 weeks shorter than normal On the other hand they also show some signs of relative immaturity (5 13 14) Abnormal serum protein patterns have been described in these infants Sirek and co-workers found by paper electrophoresis an elevated level of α_2- and β-globulin (16) and additional precipitin arcs were demonstrated by immunoelectrophoresis (17 18) By agarose gel electrophoresis of serum proteins in newborn infants of diabetic mothers the present author found an elevated concentration of β_1-globulin (transferrin) whereas total protein and the remaining electrophoretic fractions were a little though not significantly lower than in infants of non-diabetic mothers of similar gestational age and mode of delivery (4)

By zone electrophoresis of serum proteins the fractions are mainly determined by the quantitatively dominant proteins albumin α_1-antitrypsin α_2-macroglobulin transferrin and γ-G-globulin and little or no information about the smaller fractions can be obtained A large number of quantitatively small but functionally important specific globulins in serum are determinable by immunological methods In newborn infants immunological determination of a few specific globulins has been described by several authors (7 10 11

glycaemic infants indicating that SGA babies born to non toxaemic mothers do not have the same tendency to develop hypoglycaemia as when toxaemia is present

Routine blood glucose screening in all new born infants at risk should always be carried out for several days Even full term newborns with a normal birth history may develop hypoglycaemia and close observation in neonates of all categories is therefore necessary Even minor symptoms should prompt a blood glucose estimation in order to detect hypoglycaemia as early as possible and before serious symptoms occur

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Table 1 *Concentration of serum globulins in newborn infants of non-diabetic mothers (vaginal delivery)*

	Gestational age				Reference serum Adults
	<31 weeks	31–34 weeks	35–38 weeks	39–42 weeks	
Number of infants	5	14	4	49	
Mean gestational age days	205	230	260	283	
Mean birth weight, grams	1 412	076	2 577	3 548	
Prealbumin mg/litre	60±7	63±16	83±42	105±28	285
α_1-Antitrypsin, mg/litre	1 510±571	1 645±453	1 966±458	1 703±360	2 190
Orosomucoid, mg/litre	37±46	114±104	160±114	169±72	725
α_1-Lipoprotein, % of reference	56 8±28 2	78.6± 1 6	84 0±18 9	74 4±15 1	100
α_1-E p-Glycoprotein % of reference	17 4±6.9	25 9±8.8	34.0±10.2	37 7±12.3	100
Antichymotrypsin, % of reference	13 0±28.5	34 1±47.9	39 5±25.5	37.3±19.[illegible]	100
Inter-α-trypsin-inhibitor % of reference	41 8±7 2	52.4±11 1	63 0±15.9	62.6±13.3	100
α_2-Macroglobulin, mg/litre	1 622±759	874±931	3 704±871	3 493±721	2 870
α_2-HS Glycoprotein, mg/litre	678±297	61 ± 47	530±138	416±111	600
Gc-Globulin, mg/litre	152±35	196±52	208±55	202±54	320
Unidentified α_2-Globulin, % of reference	128 2±70 4	110.3±58.5	98.3±41.2	74.3±28.8	100
Transferrin mg/litre	734±291	984±356	1 483±401	1 452±332	2 530
$\frac{\alpha_2\text{-Macroglobulin}}{\alpha_2\text{-HS-Glycoprotein}} \times 100$	47.8±57 7	503 4±177 9	718 7±152.[illegible]	863 9±163.2	

analysis was performed. The concentration of serum globulins was determined by a micromodification of crossed immunoelectrophoresis, a method originally described by Laurell (9) and technically developed for quantitative determinations by Clarke & Freeman (3). The first dimension electrophoresis was run at 25 volts per cm for 30 min in a gel of 1% agarose in diemal buffer pH 8 6. The applied serum volume was 0 4 μl. The second dimension electrophoresis was performed on glass slides 60 by 76 mm in a gel containing 10% rabbit immunoglobulin against human serum proteins (Batch No. 0568 Dakopatts A/S Copenhagen Denmark). The globulins were identified as displayed in Fig 1 by their electrophoretic mobility by comparison to a commercial standard serum (Behringwerke AG Marburg-Lahn, Germany) and by investigation with specific antisera (Behringwerke AG) against orosomucoid, antichymotrypsin, Gc-globulin, inter-α-trypsin inhibitor coeruloplasmin, α_2-HS-glycoprotein, hemopexin β_1-c-globulin, β_1-ac globulin and β_2-glycoprotein. By autoradiography after addition of radioactive T (12), the unidentified α_2-globulin was found not to be identical with thyroxine-binding globulin

By analyses of dilution series of the above-mentioned standard serum a close correlation (r=0.99) was found between the height of the precipitated peak and the concentration of each globulin. The concentrations were calculated as mg/l by use of the regression equation. For orosomucoid the correlation was not rectilinear in the lower range and thus a standard curve was used for the determination of this globulin. For globulins not present in the standard serum the concentration was calculated as a percentage of a reference serum pool, collected from 77 healthy women aged 19 to 46 years (none were pregnant or taking contraceptive steroids). The coefficient of variation was between 5 and 15% for the referred globulins. The reported concentrations are the arithmetical mean values±standard deviation for the groups. Statistical standard methods were used (2).

RESULTS

The mean values for gestational age birth weight and concentration of serum globulins are presented in Tables 1 and 2 In the literature immunological determinations of serum proteins are often expressed as a percentage of a reference serum pool and to facilitate comparisons the concentrations of the present reference pool are given in Table 1

The ratio α_2-macroglobulin/α_2-HS-glycoprotein×100 was calculated for each infant and the mean values indicated in the tables This ratio would be expected to be independent of varying degrees of hydremia in the infants which e g hamper comparisons between infants delivered vaginally and those born by cesarean section (4 8 11)

Infants of non-diabetic mothers

These infants all delivered vaginally were separated into 4-week groups according to their gestational ages (Table 1) In infants born at term the concentrations of all glob-

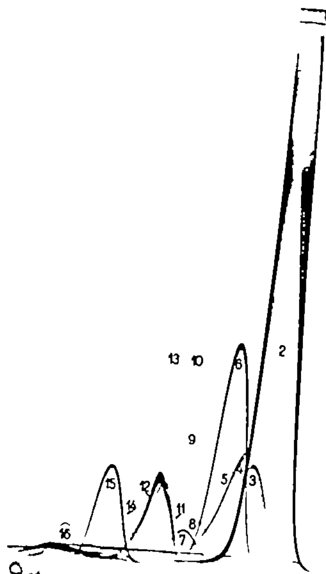


Fig 1 Quantitative immunoelectrophoresis of a cord blood serum 1 Prealbumin 2 Albumin 3 Orosomucoid 4 α Lipoprotein 5 α-cp-Glycoprotein 6. α_1-Antitrypsin 7 Antichymotrypsin 8. Inter-α-Trypsininhibitor 9 GC-globulin 10 Unidentified α_2-globulin 11 Coeruloplasmin, 12. α_2-Macroglobulin, 13 α_2-HS-Glycoprotein 14 Hemopexin 15 Transferrin, 16 β_1-ac-Globulin

9) and of a greater number of globulins)rams & Freeman (1) and most recently anrot (6) Generally speaking a lower ntration of the globulins as compared adult serum has been reported apart α_2-macroglobulin which is found at an or higher concentration In premature the concentration of a number of im-)gically determined proteins has been o be positively correlated to the gesta ge (19)

aim of the present study was to in- e the serum globulins by crossed im- ectrophoresis (3) in newborn infants tic mothers and in a reference group with a special regard to the correlation be tween globulin concentration and the gesta tional age of the infants

MATERIAL

The investigation comprised 40 newborn infants of diabetic mothers delivered at the Royal Maternity Department B Rigshospitalet Copenhagen 9[illegible] newborn infants of mothers without glucosuria or other signs of diabetes, delivered vaginally were investigated as a reference group For a detailed clinical description of the material, see a recent paper concerning largely the same infants (5)

METHODS

Mixed cord blood was collected from the placental end of the umbilical cord Serum was stored at −18°C until

Table 3 *Coefficients of correlation (r) between gestational ages and globulin concentrations in infants of nondiabetic (n=43) and diabetic mothers (n=27)*

	Infants of non-diabetic mothers Vaginal delivery	Infants of diabetic mothers Cesarean section
Prealbumin	0.465***	0.266
α_1-Antitrypsin	0.346	−0.016
Orosomucoid	0.414	0.075
α_1-Lipoprotein	0.286	0.157
α_1-E-β-Glycoprotein	0.541**	0.102
Antichymotrypsin	0.282	0.078
Inter-α-trypsin-inhibitor	0.423	0.158
α_2-Macroglobulin	0.617**	0.285
α_2-HS-Glycoprotein	−0.530***	−0.219
Gc-Globulin	0.268	0.173
Unidentified α_2-Globulin	−0.402**	−0.042
Transferrin	0.6[illegible]4**	0.09?
——— ×100 / α_2-HS-Glycoprotein	0.761	0.560*

Gestational ages <39 weeks
Infants born at term included (n=9?)
-values significantly different from zero are marked ($p<0.05$) or ** ($p<0.001$).

betes group this ratio showed the only significant correlation

In infants of non-diabetic mothers the birth weight was correlated to the globulin concentrations in nearly the same way as the gestational age In the diabetes group no correlation was found to the increased birth weight not even for the α_2-macroglobulin/α_2-HS-glycoprotein ratio ($r=0.041$ $p>0.05$)

Other globulins

Haptoglobin hemopexin and coeruloplasmin were only detectable in a small number of the infants and were not taken into the calculations or the tables The occurrence and concentrations of these proteins in infants of diabetic mothers did not differ from the reference group γ-G-globulin and β_1-ac-globulin were present in all infants but quantitation was not possible because a well defined peak was not developed

By investigation of serum pools from the groups the quantitative differences expected from the mean values were found but in serum pools the α_1-lipoprotein peak had a more cathodic mobility and in the diabetes group this peak was apparently split up in two

DISCUSSION

Infants of non-diabetic mothers

The globulin concentrations found in cord blood of term infants are in agreement with previous reports (1 6 10 11 19) Thom et al (19) reported a positive correlation between gestational age and a number of proteins with r values close to those reported here

The negative correlation between gestational age and α_2-HS-glycoprotein or other α_2-globulins has not previously been observed The observation of this negative correlation is the more important as it excludes variations in protein concentrations as being merely the result of decreasing hemodilution, and it permits by calculating the ratio between a positively and a negatively correlated globulin to compare the globulin concentrations to the gestational age independent of variations in the degree of hydration of the newborn as exemplified in this report by the calculation of the α_2-macroglobulin/α_2-HS-glycoprotein index

Infants of diabetic mothers

When differences in gestational age and mode of delivery were considered most of the globulin concentrations in the infants of diabetic mothers were comparable to those in infants of non-diabetic mothers However a lower concentration of α_1-lipoprotein and α_2-macroglobulin was observed in all groups of infants of diabetic mothers Moreover a higher concentration of transferrin was present in all of the infants of mothers belonging to White's group B–F but not present in group A

The apparent duplication of the α_1-lipoprotein peak in serum pools from infants of diabetic mothers is in accordance with the ob-

Table 2 *Concentration of serum globulins in new born infants of diabetic mothers*

	Infants of diabetic mothers		Infants of non-diabetic mothers
	Cesarean section	Vaginal delivery	Vaginal delivery
Number of infants	24	10	24
Mean gestational age days	259	261	260
Mean birth weight grams	3 706	3 565	2 577
Prealbumin mg/litre	76±24	77±13	83±42
α_1-Antitrypsin mg/litre	1 636±389	1 900±441	1 966±458
Orosomucoid mg/litre	122±74	178±138	160±114
α_1-Lipoprotein % of reference	68.5±22.6	60.7±14.1	84.0±18.9
α_1-E-p-Glycoprotein % of reference	34.1±14.4	41.1±13.9	34.0±10.2
Antichymotrypsin % of reference	22.6±17.1	31.9±28.0	39.5±25.5
Inter-α trypsin-inhibitor % of reference	56.0±17.7	68.2±16.6	63.0±15.9
α_2-Macroglobulin mg/litre	2 534±999	3 010±718	3 704±871
α_2-HS-Glycoprotein mg/litre	593±208	634± 65	530±138
Gc-Globulin mg/litre	195±74	239±59	208±55
Unidentified α_2-Globulin % of reference	79.8±43.1	10[illegible].9±37.6	98.3±41.2
Transferrin mg/litre	1 630±470	2 017±782	1 483±401
$\frac{\alpha_2\text{-Macroglobulin}}{\alpha_2\text{-HS-Glycoprotein}} \times 100$	434.1±132.2	548.3±244.0*	718.7±152.2

Gestational ages 35 to 38 weeks. Separated according to mode of delivery and compared with infants of non-diabetic mothers of the same gestational age
* Significantly different ($p<0.05$) from the non-diabetic group

ulins apart from α_2-macroglobulin were lower than in adult serum The concentration of most globulins showed a steady rise up to the 39th gestational week during the last weeks the level was constant or even falling a little α_2 HS-glycoprotein and the unidentified α_2-globulin on the contrary appeared to be negatively correlated to the gestational age

Infants of diabetic mothers

The majority of these infants were born in the thirty fifth to thirty-eighth gestational weeks Table 2 compares these infants with infants of non-diabetic mothers of the same gestational age In the vaginal delivery groups a significantly lower α_1-lipoprotein and α_2-macroglobulin concentration and a significant lower α_2 macroglobulin/α_2-HS-glycoprotein ratio were found in the infants of diabetic mothers The transferrin concentration was significantly higher in the diabetes group

In infants of diabetic mothers the concentration of most globulins was found to be 10 to 15% lower when the infants were born by cesarean section but none of these differences reached statistical significance The severity of the maternal diabetes as expressed by White s (20) classification was not found to affect the globulin concentrations in the infants except that the transferrin level was not elevated in infants of mothers with mild diabetes (White s group A) No specific globulin alterations were found in infants suffering from respiratory distress syndrome

Correlation between gestational age birth weight and globulin concentration

In infants born prematurely (gestational age <39 weeks) to non-diabetic mothers a significant correlation was found between gestational age and the concentration of most globulins For some of the globulins this correlation was still rectilinear as judged from scattergrams and the mean values also when infants born at term were included (Table 3) For α_2-HS-glycoprotein and the unidentified α_2-globulin the correlation was negative. The highest degree of correlation was found between gestational age and the α_2-macroglobulin/α_2 HS-glycoprotein ratio In the dia

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A HYDRAULIC MULTIPLE INTESTINAL BIOPSY APPARATUS FOR INFANTS

G W MEEUWISSE T LINDBERG and M BLOMBERG

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KEY WORDS: Infants, multiple intestinal biopsy apparatus

Several types of capsules for peroral intestinal biopsy in infants have been constructed (1 6 8 10) The drawbacks of these capsules are that most of them yield 1 or 2 biopsy specimens per intubation that the specimens cannot be obtained before the capsule has been removed from the patient and that they cannot be used in small infants Hydraulic devices (2 3 4 5 7) permit multiple biopsy and the specimens are successively flushed up through the tube Because of their size these instruments are however unsuitable for use in small children Since 1966, we have used a small multiple biopsy instrument which allows specimens to be obtained from the whole length of the gastrointestinal tract even in small infants The capsule briefly described previously (9) is a modified and miniaturized version of an unpublished device constructed by Lehmann

DESCRIPTION OF APPARATUS

The capsule

The capsule overall dimensions Ø 3.8 mm×9 mm (Fig 1) consists of a cylinder (1) in which a plunger (2) (Ø 3×3 mm) can move freely This plunger has a sharp edge and cuts off the mucosa projecting through the 1.65 mm hole (3) in the cylinder The end wall of the cylinder (4) has three holes and a centre pin acts as a valve which does not open until the sample is cut off The flow then flushes the sample through the tube A plastic ring (5) functions as a stop for the knife Apart from this ring, all parts are made of stainless steel (alloy SIS 2343) and are carefully ground to accurate dimensions.

The tube

The capsule communicates with the driving unit by means of two vinyl tubings secured together with cyclohexanon (Fig. 2) The tubing on the pressure side is a Portex NT 3 Shore 90, with an outer Ø mm. The first 20–30 cm however are a Portex NT-4 tubing with a larger diameter This part, which remains outside of the patient, will burst first if the flow of fluid should by chance be obstructed more distally The delivering tube (manufactured by Argyle) has a radiopaque inset and an inner Ø 2 mm and an outer Ø 3 4 mm

servations of Sirek et al (17) but the increased concentration of hemopexin reported by the same group (18) was not confirmed in this investigation

In infants of diabetic mothers the ratio α_2 macroglobulin/α_2-HS glycoprotein is significantly correlated to the gestational age and significantly lower than in infants of non-diabetic mothers As judged from this parameter infants of diabetic mothers are comparable to reference infants of about 4 weeks lower gestational age A relative immaturity in spite of an increased birth weight is also suggested in other investigations of infants of diabetic mothers they have a delayed development of ossification centres an increased extramedullary hemopoiesis and functionally an increased hyperbilirubinemia and high incidence of RDS is found (14) Recently a higher concentration of the fetal blood proteins α_1 fetoprotein (13) and hemoglobin F (5) in infants of diabetic mothers has been reported Lack of enzymes has been proposed as the cause of some of these alterations (14) and this might well be the explanation of the slightly differing protein pattern in infants of diabetic mothers

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KEY WORDS: Infants, multiple intestinal biopsy apparatus

Several types of capsules for peroral intestinal biopsy in infants have been constructed (1 6 8 10) The drawbacks of these capsules are that most of them yield 1 or 2 biopsy specimens per intubation that the specimens cannot be obtained before the capsule has been removed from the patient, and that they cannot be used in small infants. Hydraulic devices (2 3 4 5 7) permit multiple biopsy and the specimens are successively flushed up through the tube Because of their size these instruments are however unsuitable for use in small children Since 1966, we have used a small multiple biopsy instrument which allows specimens to be obtained from the whole length of the gastrointestinal tract even in small infants The capsule briefly described previously (9) is a modified and miniaturized version of an unpublished device constructed by Lehmann

DESCRIPTION OF APPARATUS

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The capsule overall dimensions Ø 3 8 mm×9 mm (Fig. 1) consists of a cylinder (1) in which a plunger (2) (Ø 3×3 mm) can move freely This plunger has a sharp edge and cuts off the mucosa projecting through the 1 65 mm hole (3) in the cylinder The end wall of the cylinder (4) has three holes and centre pin acts as a valve which does not open until the sample is cut off The flow then flushes the sample through the tube A plastic ring (5) functions as a stop for the knife Apart from this ring, all parts are made of stainless steel (alloy SIS 2343) and are carefully ground to accurate dimensions.

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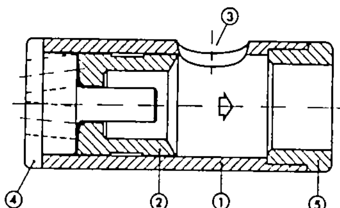


Fig 1 Cross section of the biopsy capsule Measurements Ø 3 8 mm length 9 mm *1* cylindrical body *2* plunger with sharp edge *3* biopsy port Ø 1 65 mm *4* end wall carrying central pin (not sectioned for better visibility) *5* plastic stop

The capsule is inserted into the delivering tube after extraction of the softener by immersion of the distal 10 mm of tube into aether for 20 min The bend connecting the pressure tube with the capsule is also made of polyvinyl tubing after extraction of the softener A linen ligature is applied over the capsule and covered with liquid plastic made of polyvinyl cuttings and cyclohexanon After 30 minutes in 80–90°C the hole of the capsule is exposed by cutting a round hole in the covering hardened vinyl tube

The driving unit

The main part of the apparatus is a pneumatically driven pressure amplifier (*C*) producing a hydraulic pressure of about 20 kp/cm² (Fig 3) A stainless steel cylinder with a high pressure gasket is mounted on top of a 50 mm diameter standard pneumatic cylinder (Mecman 1500/50) The chromeplated plunger shaft acts as a plunger in this cylinder The stroke volume is 15 ml The cylinder is operated by a standard 4-way pneumatic valve (V 1) Standard valves (Mecman) also control the flow from the fluid store and to the waste-bottle A removable filter is inserted before V 4 to protect the valve against solid particles

To avoid corrosive effect of saline a sodium phosphate-buffer solution (dihydrophosphate 0 018 M and monohydrophosphate 0 077 M) is used as the hydraulic medium

To produce the necessary vacuum to make the intestinal tissue enter the hole in the capsule an air driven ejection sucker (AGA JR 1800) (*E*) and two over flow valves are used The vacuum will usually be set to 0.3–0 4 kp/cm² The knife of the capsule must remain in retracted position during this period Thus the vacuum on the pressure side of the capsule (to the left in the figure) has to be higher (0 1 kp/cm²) Once established the vacuum will remain constant without using the sucker The function is fully automatic the pressures being indicated on two manometers

All parts of the driving unit are built into a light metal box dimensions 200×240×300 mm total weight 10 kg (Fig. 4) On top of the box is mounted a standard 20 ml syringe with a metal plunger where the specimens are collected The bore of the nipple is enlarged to Ø 2.5 mm The tubings are connected to the unit with the locking nuts on the top surface

The unit is connected to the central compressed air installation or to an air- or CO_2-flask The pressure must be reduced to 4.5 kp/cm²

Procedure

Infants and children were intubated through the mouth if less than 4–6 months otherwise through the nose after at least 4 hours fasting. About half of the children were premedicated with alimemazine 2 mg per kg body weight orally and/or with phenobarbital 30 mg in infants and 60 mg in children rectally When using the nasal route local anaesthesia was given with lidocaine 4% together with *epinephrine 1 1000 1 drop per 5 ml* lidocaine

The tube was introduced under fluoroscopic control with the aid of a Seldinger guide wire (length 1.5 m diameter 0 9 mm) lubricated with silicone grease It usually takes 10–15 minutes to reach the duodenojejunal flexure

Before biopsy the guide wire is removed and the tubes are connected to the driving unit and the whole system is filled with phosphate buffer When more than 1 biopsy specimen is taken the capsule is moved 1–2 cm between each biopsy

The biopsy tube is cleaned with 1% chlorhexidine for 1 hour thereafter it is flushed with water then with 95% alcohol and finally air is suctioned through the tube until dry

EXPERIENCE

Before use on patients the device was first tested on anaesthetized dogs and then on one of the authors

Since 1966 a total of 1 674 biopsy specimens have been taken from flexura duodeno-

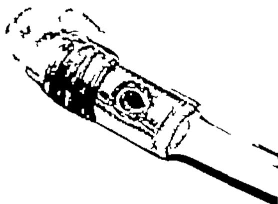


Fig 2 The biopsy mounted in the vinyl tube

jejunalis on 393 occasions. Several patients have been biopsied more than once. The youngest patient was 2 weeks old, the smallest weighed 2.2 kg. The failure rate has been low. In one patient no biopsy was obtained because the child punctured the tube with his teeth. On one occasion a destroyed specimen was flushed up and it was impossible to reopen the capsule while still in the patient. In 4 patients the intubation was interrupted through failure to pass the pylorus. In all these patients a biopsy was successfully obtained on the second attempt.

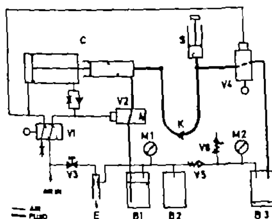


Fig. 3. Flow chart of the apparatus. *B1–3*, vacuum bottles, *C*, cylinder with pressure amplifier, *E*, ejection sucker, *K*, capsule, *M*, manometers, *V1*, 4-way valve, manual, *V2*, -way high-pressure valve, pneumatic spring return, *V3*, needle valve, *V4*, 2-way valve, manual, pneumatic return, *V5*, overflow valve, fixed, *V6*, overflow valve, adjustable, *S*, syringe for sample collection.

Function. (1) To start up, *V3* is opened to actuate the suction unit *E*, thus creating a vacuum in the bottles *B1* and *B3*. The vacuum in *B3* is determined by the setting of *V6* and is held at 0.3–0.4 kp/cm². *V5* keeps the vacuum in B1 and B2 0.1 kp/cm² higher. (2) *V4* is opened. The vacuum of *B3* will cause the tissue to enter the capsule. Excess intestinal fluid goes to *B3*. (3) *V1* is pushed. The plunger of *C* goes forward and the pressure is transmitted to the biopsy capsule. The knife of the capsule cuts off the sample which is transported to the syringe *S* as V4 has closed. The sample may now be removed. (4) When *V1* is pulled the cylinder plunger will return, V2 opens and water is sucked up from *B1*. Thus the vacuum in the bottles is maintained without using the sucker *E*. In the capsule, the vacuum causes the knife to move backwards to the position with the side-hole opened.

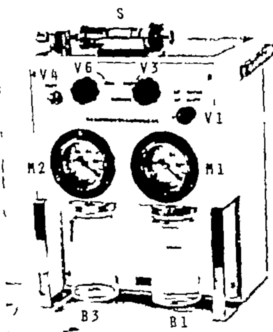


Fig. 4. Exterior of the driving unit. See legend of Fig. 3 for explanation of code.

Wear of capsules

Seven capsules of the described construction have been in use. Three of them had one side-hole, whereas the others had two side-holes. With one twin-hole capsule 650 biopsies have been taken on 170 occasions. This capsule, still in use since 1968, has never been taken apart. Two other capsules are also currently in use. Some of the first capsules did not always function satisfactorily because too much tolerance between the knife and the inner surface of the cylinder caused the knife to stick in the forward position with some fibres of muscularis mucosae between the knife and the inner wall of the capsule. A close fit of the knife in the capsule at manufacturing is therefore important.

Complications

So far only one complication has occurred. The 30th patient, a boy of 7 months who

proved to have a normal mucosa had clinical signs of intestinal perforation on the day after the biopsy He was operated on and survived without sequelae In this case 12 specimens were taken near each other After this experience it has become routine to move the capsule 1–2 cm between each biopsy Furthermore this first capsule had a larger hole Ø 2 mm In other patients as many as 20 biopsy specimens have been taken without adverse effects No gross bleeding has occurred The tube has always been easy to remove

Distant biopsy

One of the described capsules was mounted on tubes of 3 m length and a mercury bag was tied on the tip Four children and one adult were biopsied from the lower parts of the ileum or from the colon which was reached in about 2 days Because it is seldom indicated to perform long intubations in small children a somewhat larger capsule with an inner diameter of 4 mm has been constructed The tip of the tube carries an inflatable rubber balloon which can be filled with water (usually 4 ml) and emptied again before withdrawal through a third tube Seventeen patients most of them adults have been successfully biopsied with this capsule The lower parts of the ileum can be reached in 8–24 hours

COMMENTS

The great advantage with hydraulic biopsy instruments especially for infants and children is that the specimens can be obtained within a few seconds without removing the instrument from the patient

The small size of the capsule the small hole in the capsule and the flexibility of the tube make our instrument very useful even for small infants These properties also make it easier to perform biopsies in children and adults The vacuum by which the intestinal mucosa is sucked into the capsule can be regulated and controlled this increases the safety of the biopsy procedure For small infants we start with a low vacuum and increase it successively until specimens of appropriate size are obtained The vacuum is then usually about 0 3 kp/cm² The size of the biopsy specimens has proved adequate for diagnostic purposes histological examination and enzymatic analyses (disaccharidases and dipeptidases)

ACKNOWLEDGEMENTS

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PSEUDOMONAS AERUGINOSA INFECTION IN CYSTIC FIBROSIS

Occurrence of Precipitating Antibodies against Pseudomonas Aeruginosa in Relation to the Concentration of Sixteen Serum Proteins and the Clinical and Radiographical Status of the Lungs

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From the Statens Seruminstitut Department of Clinical Microbiology at Blegdamshospitalet the Paediatric Clinic TG with the Paediatric Clinic of Dronning Louises Børnehospital the Radiologic Department TX Rigshospitalet and the Protein Laboratory University of Copenhagen Copenhagen Denmark

ABSTRACT Høiby N., Jacobsen, L., Jørgensen, B. A., Lykkegaard, E. and Weeke, B. (Statens Seruminstitut, Department of Clinical Microbiology at Blegdamshospitalet, the Paediatric Clinic TG with the Paediatric Clinic of Dronning Louises Børnehospital, the Radiologic Department TX, Rigshospitalet, and the Protein Laboratory University of Copenhagen, Copenhagen, Denmark). Pseudomonas aeruginosa infection in cystic fibrosis. Acta Paediatr Scand, 63: 843, 1974.—The significance of Pseudomonas aeruginosa infection in the respiratory tract of 9 cystic fibrosis patients have been studied by means of immunoelectrophoretical analysis of patients' sera for the number of precipitins against Pseudomonas aeruginosa and the concentrations of 16 serum proteins. In addition, the clinical and radiographical status of the lungs have been evaluated using 2 scoring systems. Precipitins against Pseudomonas aeruginosa were demonstrated in all sera, the maximum number in one serum was 22. The concentrations of 12 of the serum proteins were significantly changed compared with matched control persons. Notably IgG and IgA were elevated and the "acute phase proteins" were changed, the latter suggesting active tissue damage. The concentrations of 3 of the acute phase proteins, notably haptoglobin were correlated to the number of precipitins suggesting that the respiratory tract infection in patients with many precipitins is accompanied by more tissue damage than the infection in patients with few precipitins. The results indicate no protective value of the many precipitins on the tissue of the respiratory tract.

KEY WORDS: Cystic fibrosis, pseudomonas aeruginosa, serum proteins, immunoelectrophoresis

Patients suffering from cystic fibrosis (CF) often harbour Pseudomonas aeruginosa (Ps aeruginosa) especially mucoid strains in the respiratory tract (4 5) Høiby & Axelsen (7) have shown that these patients regularly produce precipitating antibodies against a multivalent Ps. aeruginosa antigen and up to 50 different precipitins against Ps aeruginosa have been demonstrated in one serum (9) Eradication of the infection from the respiratory tract is however not effected and many CF patients harbour Ps aeruginosa in the respiratory tract for years simultaneously producing many and strong precipitins against these bacteria (8)

The aim of this work was to study the significance of Ps aeruginosa infection in CF patients To evaluate the significance of this infection CF patients harbouring solely Ps aeruginosa in the lower respiratory tract

were simultaneously examined for 1) the occurrence and number of Ps aeruginosa precipitins 2) the concentrations of 16 serum proteins with special regard to the acute phase proteins (1 17) 3) X-ray pictures of the chest were evaluated and 4) the clinical status of the patients lung disease were evaluated

MATERIALS AND METHODS

Patients

Nine CF patients were included in the study They were selected among 33 CF patients who at the same time were examined for the occurrence of Pseudomonas precipitins (7) Only these patients could comply with the demands of this study 1) The patients should have harboured Ps aeruginosa in the respiratory tract for at least 1 month previous to the study and 2) no other bacteria should be present in the lower respiratory tract at the time of this study or the preceeding ? months. All patients have been followed as out-patients for at least 6 months including monthly bacteriological examination of sputum or tracheal secretion obtained by aspiration through a catheter as described previously (7) In Table 1 sex and age distributions are given as well as the results of the bacteriological examinations as regards Ps aeruginosa

Controls

Nine healthy children matching the CF patients with regard to age and sex were included in the study to serve as controls concerning the concentrations of serum proteins. These controls were randomly selected from matching normal children included in a previous work (16)

Ps aeruginosa precipitins

The number of circulating Ps aeruginosa precipitins in the patients sera was examined by means of a polyvalent Ps. aeruginosa antigen (St Ag) using a modified crossed immunoelectrophoresis method as described previously (7)

Examination of 16 serum proteins

Serum albumin IgG IgA and IgM were determined by means of the rocket immunoelectrophoresis (18) using rabbit antiserum against human albumin –IgG –IgA –IgM (Dakopatts Copenhagen) The remaining 12 serum proteins were identified and quantitated by means of crossed immunoelectrophoresis in microtechnique (19) using a rabbit antiserum against human serum proteins (Dakopatts Copenhagen) By comparison with a standard serum (Behringwerke, West Germany) the results were expressed in g/l. Antichymotrypsin was expressed in arbitrary units (U/l) by comparison with a normal 1 000 donor serum pool (=100 U/l).

Radiological assessment

Analysis of the chest radiographs was carried out in correspondance with Norman's (14) system. According to this, chest configuration is assessed by evaluating the degree of sternal bowing, spinal kyphosis and diaphragmatic depression Pulmonary changes deal with line shadows mottled shadowing ring shadows and large pulmonary shadows and are assessed separately for 4 zones of the lungs (right upper and lower zone left upper and lower zone) Line shadows are straight line branching patterns radiating from the hilar regions. Mottled shadowing is multiple small rounded shadows with ill-defined edges. Ring shadows are formed by central area of increased lung transradiency circumscribed by a descrete shadow of less transradiency Large pulmonary shadows are associated with collapse and/or consolidation of a lobe or a segment of a lobe. All changes are graded according to severity (0– points each) and in the present study all points were added leaving a final score of 0–38 points (X-ray score), the most disabled patients having the highest score

Clinical assessment

The clinical severity of the lung disease was assessed by a simple scoring system paying attention to the following 5 factors, cough sputum crackling lung sounds, dyspnoea/cyanosis and clubbing of the fingers. The first 3 signs were given 0–3 points each according to severity 2 points were given if dyspnoea and/or cyanosis was clearly present if not 0 points were given and 1 point was given if clubbing was clearly present. All points were added leaving a final score of 0–1 points.

Statistical methods

The Wilcoxon test for pair differences and Spearman's correlation coefficient R (3)

The results of each of the parameters were obtained blindly with respect to knowledge of the results of any of the other parameters

RESULTS

In Table 1 the results for each of the 9 CF patients are given It is seen that a wide range of scores and number of precipitins were reached by the patients There was no relationship between the age of the patients or the duration of the Ps aeruginosa infection and 1) the X-ray score 2) the clinical score and 3) the number of Pseudomonas precipitins Nor was there any relationship between the number of Ps aeruginosa precipitins and the X-ray score or the clinical score

Table 1 *Sex and age distribution of 9 cystic fibrosis patients and the number of Pseudomonas aeruginosa (Ps aer) precipitins X-ray scores and clinical scores*

Each patient is indicated by a number The duration of the Ps aer infection is gi en as well as the type of strain isolated at the time of the study For explanation of the scores see text

Patients	Age	Sex	Duration of Ps aer infection (years)	Mucoid (M) or nonmucoid (NM) Ps aer strain	No of Ps aer precipitins	X-ray score	Clinical score
1	16	F	1/?	M	4	7	8
	8	M	5/6	M	14	~6	11
3	5	F	1/1	NM		13	9
4	10	F	>1/?	M	5	15	9
3	1	M	>	M	15	18	9
6	11	F	1 3/4	M	5	4	5
7	6	M	2	M	18	13	7
8	11	M	7/12	M	22	17	1
9	5	M	5/6	NM	8	4	5

The X-ray score was positively correlated to the clinical score (Fig. 2)

In Fig. 1 *a* and *b* the concentrations of the single serum proteins in the CF group and the control group are given Significantly increased values of the following serum pro-

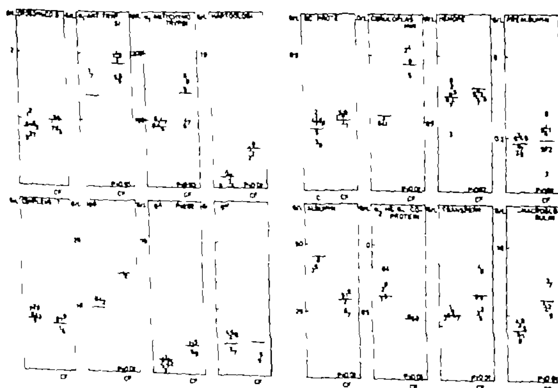


Fig 1 (*a b*) The concentrations of 16 serum protein in 9 cystic fibrosis patients (*CF*) and 9 matching control persons (*C*) The bars represent the mean values. The *p* values indicate the probability of statistically significant differences between the groups. The concentrations are given in grams per litre serum (G/l) or arbitrary units per litre serum (U/l) compared to a pool of normal sera (=100 U/l) Each patient and his matching control are indicated by a number corresponding to Table 1

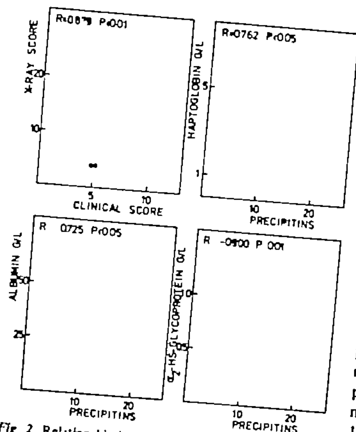


Fig 2 Relationship between *1)* X-ray score and clinical score and 2) the number of Pseudomonas precipitins (precipitins) and the concentrations of haptoglobin albumin and α_2-HS-glycoprotein The concentrations are given in grams per litre serum (G/l) For explanation of the scores see text Spearman s correlation coefficient (*R*) and the corresponding probability of statistical significance (*p*) are indicated in the figure

teins were found in the CF group α_1 antitrypsin α_1 antichymotrypsin haptoglobin ceruloplasmin hemopexin prealbumin transferrin α_2 macroglobulin IgG and IgA Significantly decreased values of albumin and α_2-HS-glycoprotein were found in the CF group

The values of the 12 serum proteins which differed significantly from the control values were tested for correlation with 1) the number of Ps aeruginosa precipitins 2) the X ray score and 3) the clinical score The concentration of haptoglobin was positively correlated to the number of Ps aeruginosa precipitins whereas the concentrations of albumin and α_2-HS-glycoprotein were negatively correlated to the number of Ps aeruginosa precipitins No correlation was found between the concentrations of any of the serum proteins and the X-ray score or the clinical score

DISCUSSION

Most CF patients are subjects to recurrent and chronic respiratory tract infections (5 and these patients frequently harbour several different bacterial species simultaneously in the lower respiratory tract In this study it was only possible to include 9 CF patients among which Ps aeruginosa could be regarded as the only possible cause of infection at the time of this investigation and the preceeding 2 months

From Fig. 1 *a* and 1 *b* it appears that CF patients harbouring Ps aeruginosa differ from matching controls with respect to many serum proteins Especially the concentrations of many acute phase proteins (1 10–13 15 17) are significantly changed (concordantly increased α_1 antitrypsin α_1-antichymotrypsin haptoglobin ceruloplasmin and hemopexin concordantly decreased α_2 HS-glycoprotein and albumin) This type of correlated alterations in the acute phase proteins are generally found under circumstances where tissue damage takes place (1 10–13 15 17)

The concordantly elevated IgG and IgA concentrations in CF patients is not surprising considering the chronic infections of the patients

The reason why transferrin prealbumin and α_2-macroglobulin were increased are obscure There were no signs of sideropen anemia respectively uremia nephrosis diabetes mellitus or hormonal disturbances or treatment which could be responsible for these changes

The clinical score was correlated to the X-ray score (Fig. 2) Both these scores reflect the combined effects of active processes as well as former damage to the tissues This could be the reason why the scores were not correlated to the concentrations of the serum proteins because especially the acute phase proteins reflect active tissue dam-

age. This might also be some of the explanation why the scores were not correlated to the number of Ps. aeruginosa precipitins

The correlations found between the number of Ps. aeruginosa precipitins and the concentrations of some of the serum proteins which participate in the "acute phase reaction" especially haptoglobin are interesting. It has formerly been shown that the concentration of haptoglobin is especially well positively correlated to lung diseases (10–13 15 17) The present findings therefore suggest that the respiratory tract infection in CF patients with many precipitins is accompanied by more tissue damage than the infection in patients with few precipitins

Our findings of changed "acute phase proteins" are in accordance with the clinical impression (5) that Ps aeruginosa infection in the respiratory tract of CF patients is a main factor in the pathology of the tissue damage The correlations between the number of precipitins and some of the "acute phase proteins" are also in accordance with Doggett & Harrison (5 6) who found that high and rising titres of Ps aeruginosa antibodies can be a sign of poor prognosis in CF patients—considering the positive correlation between the number of precipitins and the titres of the strongest precipitins (7 8)

The many Ps aeruginosa precipitins seems defective with respect to protection against progression of the tissue damage or eradication of the infection in the lungs of CF patients In fact one could speculate whether the high number of precipitins and the persistent infection by means of a type III hypersensitivity reaction (2) could possibly contribute to the tissue damage in the lungs of CF patients On the other hand these antibodies possibly play a role in localizing the infection to the respiratory tract as these patients rarely if ever get generalized infection caused by Ps aeruginosa (5 8)

Hoiby & Axelsen (7) have recently suggested that the defective protection of the lung tissue offered by the many Ps aeruginosa precipitins might—at least partly—be explained by properties of the Ps aeruginosa strains found in these patients i e production of great amounts of mucoid substance (4–6) This substance presumably inhibits the opsonizing effect of antibodies as well as the complement dependent lysis of bacterial cells in the respiratory tract of CF patients and it protects the bacteria during phagocytosis against the enzymes of the phagocytes (6) It is in accordance with this suggestion that mucoid strains are more pathogenic than nonmucoid strains of Ps aeruginosa in CF patients (5 6 8)

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FAILURE TO THRIVE IN LEBANON

IV *Longitudinal Health and Growth Data*

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ABSTRACT Kanawati, A. A., McLaren, D. S. and Darwish, O. (Nutrition Research Program, School of Medicine, American University of Beirut, Beirut, Lebanon). Failure to thrive in Lebanon IV Longitudinal health and growth data. Acta Paediat Scand, 63:849, 1974.—Pre-school age Arab children of low socio-economic standard living in urban and suburban areas of Lebanon were divided into Thrive and Failing to Thrive groups according to an Index of Thriving. They were matched for age and their young siblings were also studied. Various somatic measurements were made and health-related data collected on three occasions over a period of approximately three years. Notable among the findings were (1) the very low mortality rate, (2) evidence of considerable spontaneous catch-up growth in the Failing to Thrive group, (3) a high rate of obstetric experience in both groups, greater in the Failing to Thrive, and (4) frequent occurrence of infections with a tendency to delay vaccination, especially in the Failing to Thrive group, as a probable consequence.

KEY WORDS: Social paediatrics, failure to thrive

In this final paper in the series on failure to thrive in Lebanon we report the results obtained on two follow-up visits to the study families as they relate to the health and growth of the children.

MATERIAL AND METHODS

The study areas, *Bourj el Barajne* and *Basta* in suburban and urban Beirut have been described previously (6). Details of the method of separating children into the two contrasting groups "Thriving" and "Failing to Thrive" based on their attainment of weight, height, head circumference and mid-arm circumference in relation to international standards has been given in a previous paper (5).

In all three visits were paid to the homes of the study children. The first visit occurred some time between December 1967 and December 1968. Socio-economic and other data obtained at this time have been presented previously (6). They revealed the multifactorial nature of the phenomenon of failure to thrive in these communities. The second visit was made one year after the first (to within one or two weeks). An attempt was made to visit each of the families seen at the first visit and to re-examine every child. After a further period of approximately 2 years a second follow-up visit was paid and again as many of the families as possible were examined. Young siblings were also measured at each visit. A questionnaire was completed at each visit concerning the care and feeding of the child, disease experience and vaccination of the child, pregnancy, delivery and abortions of the mother. For each child and one sibling the following measurements were registered: weight, height, head circumference, mid-arm circumference, chest circumference, and triceps skinfold thickness. To facilitate comparison between the "Thriving" and "Failing to Thrive" groups the two groups were matched for age as was done previously for other data (6). Consequently 27 children in each group could be matched for age with a mean age of 17.2 months at the first visit (15 boys and 12 girls in the "Thriving" group and 14 boys and 13 girls in the "Failing to Thrive"). The longitudinal growth and health data reported here were confined to these two groups. The same was done for siblings of the "Thriving" group and the siblings of the "Failing to Thrive" group and 18 siblings in each group could be matched for age.

Table 1 *Number of children examined on 3 occasions by group and location*

Examination	1	2	3
Basta			
Thriving	25	25	19
Failing to thrive	18	16	14
Bourj el Barajne			
Thriving	45	39	31
Failing to thrive	37	34	24 (1 died)
	125	*114*	*88*

RESULTS

Mobility and mortality

Table 1 shows the numbers of children examined in the two areas in each nutritional group and on each of the three visits Of the 125 children seen at the first visit 114 could be re-examined one year later on the second visit the remaining 11 children had moved away from the area to unknown addresses and as far as was known had not died On the third visit about 2 years later there was a further loss of 26 cases and all of these except one had moved away and were known not to have died One child in the Failing to Thrive group of Bourj al Barajne was known to have died at about 5 years of age from an unknown cause Thus out of 125 children (70 Failing to Thrive and 55 Thriving) followed over a period of about 3 years only 1 child (of the Failing to Thrive group) was known to have died

Growth data

In Tables 2 and 3 are shown various physical measurements for the two nutritional groups and their siblings on the three visits expressed as percentages of international standards for weight height head circumference mid arm circumference chest circumference and triceps skinfold thickness The Index of Thriving previously defined and used as the basis for division into Thriving and Failing to Thrive groups (6) is also included

In the Thriving group weight % height % and mid arm circumference % declined slightly with age and this is reflected in the steady rise in the Index of Thriving Chest circumference remained somewhat above standard and triceps skinfold rose strikingly In general the Thriving group siblings showed similar trends The Failing to Thrive group showed considerable catch-up of growth of weight head circumference and mid-arm circumference but stationary height reflected in the improving Index of Thriving The triceps skinfold again increased markedly The Failing to Thrive siblings showed some deterioration in most measurements and had consistently lower measurements than the siblings of the Thriving group

Table 2 *Mean values of growth data for Thriving and Failing to Thrive groups on 3 examinations expressed as percentages of international standards and the Index of Thriving*

	Thriving group			Failing to Thrive		
	1st exam	2nd exam	3rd exam	1st exam	2nd exam	3rd exam
Age	17 [illegible]	[illegible]9 4	60 8	17 [illegible]	[illegible]9 3	60 0
Weight %	105 6	103 7	96 3	70 5	79 3	81 4
Height %	100 9	98 5	96 9	90 7	90 4	90 [illegible]
Head circ %	99 4	98 6	100 [illegible]	92 0	91 3	95 6
Mid-arm circ %	101 9	101 7	96 [illegible]	80 9	88 [illegible]	88 7
Chest circ %	104 6	104 3	104 6	91 8	95 1	98 1
Triceps skinfold %	89.5	95 5	111 4	71 3	8[illegible] 9	101 3
Index of Thriving	1 5	2 3	9	9 4	7 3	6.7

Level of significance between means of data in the three examinations is at least $p<0.05$ Those data that have the same letter are not statistically different.

Table 3 *Mean values of growth data for Thriving and Failing to Thrive sibling groups at 3 examinations expressed as percentages of international standards and the Index of Thriving*

	Thriving sibling			Failing to Thrive sibling		
	1st exam.	2nd exam	3rd exam	1st exam.	2nd exam	3rd exam
Age	32.9	45.1	77.3	33.0	45.0	76.6
Weight %	93.4	93.0	89.1	87.3	87.1	82.0
Height %	94.0	94.0	93.8	93.0	92.2	91.5
Head circ. %	96.8	97.0	98.0	95.4	95.9	97.2
Mid-arm circ. %	99.2	99.3	92.5	93.4	93.6	89.9
Chest circ. %	98.8	102.0	97.4	100.5	100.3	100.4
Triceps skinfold, %	94.1	102.6	97.6	89.7	91.3	97.9
Index of Thriving	4.2	4.1	4.8	5.2	5.6	6 .

Level of significance between means of data in the three examinations is at least $p<0.05$. Those data that have the same letter are not statistically different.

Health data

These are shown in Tables 4 and 5. The greater mobility of the Failing to Thrive group is again shown as it was in Table 1 but this time for those who could be traced and re-examined twice. The total obstetric experience of the mothers in both periods was nearly 50% greater in the Failing to Thrive group. Respiratory infections and measles were very common in both groups. Gastro-enteritis and pertussis were more common in the Failing to Thrive group. Vaccination appeared equal in frequency in the two nutritional groups and the sibling groups. However, for the Failing to Thrive vaccination tended to be later (between the 2nd and 3rd examinations rather than prior to the 1st examination). The significance of this observation will be commented upon in the discussion.

DISCUSSION

Our previous papers in this series and studies in several other parts of the world (3–11) have shown the complex and multifactorial nature of the problem of malnutrition in childhood. There are however few similar longitudinal studies with which our data may be compared. Adrianzen et al (1) recently reported anthropometric data for over 400 children of very poor slum families of Lima, Peru from which at least one child had been hospitalized because of severe malnutrition. They were measured 4 times over a period of 4 years. It is interesting to note that the main findings of these authors of better preservation of head circumference than height and the occurrence of undernutrition within families is also a feature of the present study.

In our Failing to Thrive group the head circumference at each examination was significantly lower than that for the Thriving group (Table 2). In a study of the mental development of a similar group of chronically undernourished Lebanese children and their healthy siblings tested by the Stanford Binet Intelligence Test, a slight though significant difference was shown by us (8) and a recent report from Chile concerning large numbers of undernourished children provided similar findings (9).

In the present study the catch-up growth shown for most measurements in the Failing to Thrive group is especially noteworthy. It occurred in the absence of any really effective health care in the areas and no health interventions were made by the investigators. This strongly suggests that there

Table 4 *Family and health data for Thriving and Failing to Thrive groups during t periods as percentage incidence*

	Thriving (27)			Failing to Thrive (27)		
	Up until 1st exam	Between 1st and 2nd exam	Between 2nd and 3rd exam	Up until 1st exam	Between 1st and 2nd exam	Between 2nd and 3rd exam
Previous illness						
Measles	33.3	25.9	29.6	37.0	14.8	44.4
Whooping cough	0	0	3.7	14.8	3.7	3.7
Gastro-enteritis	66.7	40.7	11.1	81.5	70.4	0
U Resp infection & other diseases	85.2	77.8	33.3	63.0	85.2	40.7
Immunization						
Smallpox vaccine	22.2	0	51.9	14.8	3.7	33.3
D P T vaccine	33.3	3.7	37.0	0	7.4	55.6
Polio oral vaccine	66.7	48.2	48.2	29.6	55.6	63.0
Family follow up data						
Changed address	0	7.4	3.7	0	[illegible]	7.5
Child lives with his father and mother	100.0	96.0	96.0	100.0	91.6	91.6
Child lives with his father only	0	0	0	0	4.2	4.2
Child lives with his mother only	0	4.0	4.0	0	4	4.2
Sibling looks after the child besides the mother	28.0	36.0	40.0	29.2	21.7	37.5
Relatives look after the child besides the mother	52.0	44.0	40.0	33.3	33.3	29.2
Others look after the child besides the mother	8.0	4.0	4.0	12.5	8.3	12.5
The mother is pregnant	0	28.0	12.0	0	20.8	8.3
Child born in the period	0	20.0	48.0	0	33.3	75.0
Abortion occurred	0	0	28.0	0	12.5	29.2
Child death	0	10.0	0	0	4.2	4.2

is a spontaneous improvement in nutrition and growth after the first 2 years or so of life In the first paper in this series (5) cross-sectional data provided similar evidence for spontaneous catch-up growth which these longitudinal data now confirm We are not aware of any similar results from elsewhere but if found to be a general phenomenon the implications could be important for health protection programmes They could suggest that efforts should be concentrated on the first 2 years of life where resources are limited and under these circumstances much expenditure on Under Fives Clinics (10) may be uneconomical

The apparent very low mortality rate in the groups of the study children (Table 1) is at first surprising Data could not be obtained throughout for 36 (20 Thriving and 16 Failing to Thrive) of the 125 children seen originally There is no reason to believe that the mortality rate would be different among those who moved away as compared with those who were examined If this assumption can be made then the figure is of the same low order as those of 6 32 or 14 per 1000 for infant mortality in Beirut (2) and 12 per 1000 for infant mortality in Lebanon (12)

The considerable mobility shown in this

Table 5 *Family and health data for Thriving and Failing to Thrive sibling groups during three periods as percentage incidence*

	Thriving siblings (18)			Failing to Thrive siblings (18)		
	Up until 1st exam	Between 1st and 2nd exam	Between 2nd and 3rd exam.	Up until 1st exam	Between 1st and 2nd exam.	Between 2nd and 3rd exam
Previous illness						
Measles	61 1	22..	22..	44 4	77.8	16 7
Whooping cough	5 6	5 6	11 1	5 6	16 7	0
Gastro-enteritis	44 4	16 7	11 1	66.7	50 0	11 1
U Resp infection & other diseases	66 7	77 8	33.3	72.2	61 1	33.3
Immunization						
Smallpox vaccine	77.8	5.6	44 4	77.8	0	50.0
D P T vaccine	27.8	5 6	50 0	11 1	0	50 0
Polio oral vaccine	83.3	38 9	38 9	55 6	50 0	61 1
Family data						
Changed adress	0	11 1	5.6	0	77.8	27.8
Child lives with father and mother	100 0	100 0	100 0	100.0	94 1	94 1
Child lives with father only	0	0	0	0	0	0
Child lives with mother only	0	0	0	0	5 9	5 9
Sibling looks after the child	22 2	38 9	44 4	29 4	35 3	41 2
Relatives look after the child	38 9	27 8	16 7	35 3	29 4	17 7
Others look after the child	16 7	11 8	16.7	23 5	17 7	17 7
The mother is pregnant	0	22.2	22 2	0	23.5	11.8
The mother had baby	0	5 6	55 5	0	29 4	82 4
The mother had abortion	0	5 6	22 2	0	11 8	29 4
The mother lost child	0	5 6	0	0	5 9	11.8

study (Table 1) and commented upon in a previous part of the work (4) might make long-term health intervention programmes difficult to complete

The data in Tables 4 and 5 on care of the child and the personal observations made in collecting the data suggest that broken homes and similar problems do not play a significant role in either group The total obstetric experience of the mothers (pregnancy delivery abortion) in both groups is high and considerably higher in the Failing to Thrive group It was found in these communities (unpublished observations) that about 25% of the mothers were practising some form of birth control (mostly non-mechanical) and efforts are clearly needed to promote family planning, for the sake of the children s health

Of the common infections pertussis was more common in the Failing to Thrive group as found in the initial examination (6) and gastro-enteritis was also more common in this group in the 2nd period

The data on vaccination are especially interesting in one respect They cannot be interpreted in detail since accurate records are not kept in the clinics and the information was obtained from the mothers and was therefore subject to considerable error It is clear however that there is a marked delay in both groups in carrying out vaccination, long after the normally prescribed periods This seems to be attributable to the

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Family follow-up data						
Changed address	0	7.4	3.7	0	22.2	7.5
Child lives with his father and mother	100.0	96.0	96.0	100.0	91.6	91.6
Child lives with his father only	0	0	0	0	4.2	4
Child lives with his mother only	0	4.0	4.0	0	4.2	4.2
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Measles	61.1	22.2	22.2	44.4	77.8	16.7
Whooping cough	5.6	5.6	11.1	5.6	16.7	0
Gastro-enteritis	44.4	16.7	11.1	66.7	50.0	11.1
U. Resp. infection & other diseases	66.7	77.8	33.3	72.2	61.1	33.3
Immunization						
Smallpox vaccine	77.8	5.6	44.4	77.8	0	50.0
D.P.T. vaccine	27.8	5.6	50.0	11.1	0	50.0
Polio oral vaccine	83.3	38.9	38.9	55.6	50.0	61.1
Family data						
Changed address	0	11.1	5.6	0	77.8	27.8
Child lives with father and mother	100.0	100.0	100.0	100.0	94.1	94.1
Child lives with father only	0	0	0	0	0	0
Child lives with mother only	0	0	0	0	5.9	5.9
Sibling looks after the child	22.2	38.9	44.4	79.4	35.3	41.2
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practice of the health authorities not to vaccinate when there are signs of any acute illness As undernourished children may spend most of their early lives in this state (7) they may be deprived of the protection of vaccination indefinitely if this practice is rigidly followed

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α_1-ANTITRYPSIN DEFICIENCY

Pi Genotype ZO SO and MO

C.-B. LAURELL, T. SVEGER and C.-O. LJUNGGREN

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ABSTRACT. Laurell, C.-B., Sveger, T. and Ljunggren, C.-O. (Departments of Clinical Chemistry and Paediatrics, Malmö General Hospital, Malmö, and the Department of Paediatrics, Norrköping Central Hospital, Norrköping, Sweden). α_1-antitrypsin deficiency: Pi genotype ZO, SO and MO. Acta Paediatr Scand, 63: 855, 1974.—Of 88 300 babies screened for classical α_1-antitrypsin deficiency, samples from 98 were requested for Pi typing. Among the first 67 infants studied, one was found to have a Pi type suggesting incompatibility with the mother. The serum concentration of α_1-antitrypsin was determined with electroimmunoassay and the Pi type by acid-crossed immunoelectrophoresis. The family study produced strong evidence for the occurrence of an inherited Pi0 allele appearing as Pi genotype MO, SO and ZO with increasing degree of α_1-antitrypsin deficiency. The phenotype Pi ZZ may cover subjects of genotype Pi ZZ and Pi ZO, which may complicate prognostic studies of classical α_1-antitrypsin deficiency. However, the Z-allele seems to be at least 20 times more common than the 0 allele.

KEY WORDS: α_1-antitrypsin, proteinase inhibitor

In Sweden neonates are being screened for α_1-antitrypsin deficiency using part of the blood sent in for the Guthrie test. The study was started at the beginning of 1973 and will be continued until the end of 1974. Infants with an abnormally low level of α_1-antitrypsin are referred to the nearest children's clinic for examination, protease inhibitor (Pi) typing and liver tests. Blood from 98 infants with low α_1-antitrypsin have so far been requested for Pi typing during the first 10 months of the investigation.

This report concerns a family study on a female infant, found in this screening with apparent phenotype Pi ZZ, but whose mother initially seemed to be of incompatible phenotype Pi MM as judged from acid crossed immunoelectrophoresis.

MATERIAL AND METHODS

Serum samples (usually cooled) are mailed to our laboratory for Pi typing, arriving within 4–36 hours and stored frozen at −22°C until analysed. The condition of the sera is checked by agarose gel electrophoresis (2).

A screening test for α_1-antitrypsin deficiency using dried blood on filter papers for the Guthrie test (3) and the Pi typing system using serum have recently been described (4). The serum concentration of α_1-antitrypsin is determined with the electroimmuno assay (5). A blood donor serum pool (1000 subjects, mainly men) is regarded as 100% and used as standard. This has preliminarily been accepted as equivalent to 2.0 g α_1-antitrypsin per litre.

FAMILY HISTORY

The proband (nr 9, Fig. 1) was a baby girl with α_1-antitrypsin deficiency. She had a normal birthweight and no neonatal disorders. Clinical examination and liver tests showed nothing remarkable at 3 and 6 months of age. None of the family members examined had clinical signs of emphysema or liver disease.

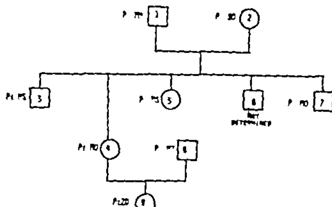


Fig 1

RESULTS

The pedigree of the proband with the proposed Pi types is given in Fig. 1 The Pi allele which corresponds to a zero concentration of α_1 antitrypsin is called Pi⁰ The Pi⁰ allele assumed by us does not influence the acid crossed immunoelectrophoretic pattern The sex age α_1-antitrypsin concentration and Pi types of the family members are summarised in Table 1 In our investigation the mean α_1 antitrypsin concentration ±S D of 42 children aged 2-3 months with Pi ZZ was 0 46±0 1 g/l

DISCUSSION

Judging from the examination of two samples drawn at 3 months interval the patient was phenotype Pi ZZ and showed an α_1 antitrypsin concentration of 0 22 and 0 20 g/l Repeated typing of the mother s (no 4) sera gave Pi phenotype MM but the α_1-antitrypsin concentration was only 1 0 g/l That is 3 3 S D below the average in healthy female Pi MM individuals The father (no 8) of the proband had 1 2 g α_1 antitrypsin per litre and Pi MZ type The proband an offspring of the mother (no 4) if Pi MM and father (no 8) with Pi MZ could never be Pi ZZ The Pi⁰ allele occurred on the mother s side

Pi typing showed the maternal grandparents to be MM and SS but two of their children (nos 4 and 7) are incompatible as offspring of parents homozygous for M and S respectively All the incompatible cases are explained if a P⁰ allele is spread in the family as suggested in Fig 1 A man with severe emphysema and no measurable α_1-antitrypsin in the serum and who may be homozygous for a Pi⁰ allele has recently been reported by Talamo et al (7) and supporting evidence has been published by Martin et al (6) and Altay et al (1)

On Pi typing the heterozygotes for the 0 allele were indistinguishable from the homozygotes for the allele with expression in the phenotype but heterozygosity should be suspected from a relatively low α_1-antitrypsin concentration in subjects with M and S alleles At 3 and 6 months of age the assumed single Z allele gave in the proband an expression in α_1 antitrypsin concentration which was below 2 S D

Owing to the existence of an inherited Pi⁰ allele subjects in the group of cases phenotyped as classical α_1 antitrypsin deficiency (Pi ZZ) may be of genotype Pi ZO The frequency of the Pi⁰ allele is more difficult to estimate than that of the alleles with expression in the crossed immunoelectrophoresis A rough estimation may however be obtained from our study of infants if it be assumed that Pi OO and/or ZO foetuses have normal intra-uterine survival The father or the mother of ZO babies with apparent ZZ

Table 1 *Serum α_1-antitrypsin concentration and Pi types in the sera studied*

Pedigree no	Sex	Age	Serum α_1-antitrypsin (g/l)	Pi type
1	M	64	3	MM
2	F	56	0 74	SO
3	M	30	1 8	MS
4	F	28	1 0	MO
5	F	27	1 8	MS
6	M	[illegible]3	0	Not determined
7	M	17	1 3	MO
8	M	28	1 2	MZ
9	F	0 25 0 5	0 22 0 20	ZO

phenotype may be recognized by their incompatible phenotype The apparently incompatible mothers will be recognized in our study

During the first 10 months of screening 18300 neonates have been studied and samples for Pi typing have been requested from 8 67 of these have been investigated 47 were Pi ZZ, 19 Pi SZ 4 Pi MZ 1 Pi MM and 1 Pi ZO If the distribution of the different Pi types is the same in the 98 infants the Z allele frequency will be 0 026 If representative the figures indicate an O allele frequency of less than 0 001

None was homozygous for the Pi° allele which lends further support to the assumption that the O allele is too rare to influence the calculation by acid-crossed immunoelectrophoresis of the Z allele frequency from observed Pi ZZ phenotypes

ACKNOWLEDGEMENT

This investigation was supported by grants from the Bank of Sweden Tercentenary Fund and the Swedish Medical Research Council (Project N B74-13X 581)

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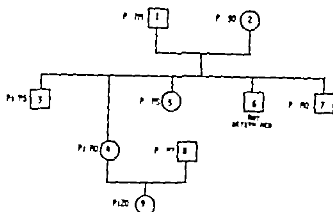


Fig 1

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8	M	28	1 2	MZ
9	F	0 25	0 22	ZO
		0 5	0 20	

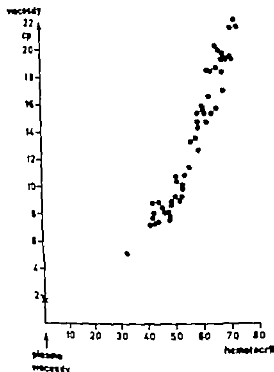


Fig 1 Whole-blood viscosity in 59 newborn infants with varying hematocrit values at shear rate 23 sec⁻¹ Plasma viscosity is also shown for comparison.

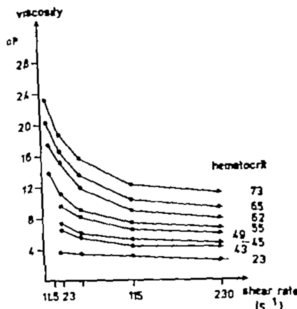


Fig 3 The variation of whole blood viscosity in 8 newborn infants at different hematocrit values and shear rates

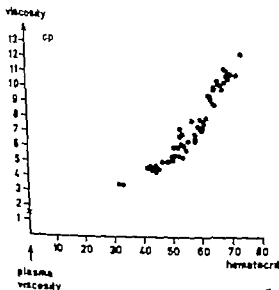


Fig 2 Whole-blood viscosity in 63 newborn infants with varying hematocrit values at shear rate 230 sec⁻¹ Plasma viscosity is also shown for comparison.

The postmenstrual age was evaluated on clinical grounds in the ordinary way (1 10 21)

No symptoms of the high hematocrit were recorded in groups B–E.

The viscosity examinations were carried out at 37.0°C with a Brookfield a LVT viscometer on blood quantities varying between 1.5 and 2.0 ml Calibration with corresponding quantity of silicon oil was regularly performed The viscosity of the silicon oil was 10 centistokes at 20°C corresponding to 9 723 cP at 37 0°C The following shear rates have ordinarily been used, 23 46 115 and 230 sec Purely methodologically the examinations were performed in the same way as described by Groth (9), Rand et al. (17) and Wells et al. (23)

No samples were kept for more than 2 hours before analysis Hematocrit determinations were made with an International Capillary Centrifuge during 6 min at 11 000 rpm. The samples were collected in heparin-flushed plastic syringes from peripheral veins, except in the case of umbilical cord blood which was collected in glass tubes with 0.2 ml heparin added to 10–12 ml blood. These tubes were centrifugated 15 min at 2 000 rpm. Plasma was separated and erythrocyte concentrate was drawn from the bottom of the tube.

RESULTS

The variation of viscosity at different hematocrit values will be seen from Figs 1–3 which show the conditions at different shear rates The first two figures show the varia-

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VISCOSITY OF THE BLOOD IN THE NEWBORN INFANT

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ABSTRACT Bergqvist G (Department of Paediatrics, Karolinska Institutet St Göran's Hospital for Children, Stockholm Sweden) Viscosity of the blood in the newborn infant. Acta Paediatr Scand, 63:858, 1974.—Whole blood viscosity has been measured with a Brookfield LVT viscometer on newborn infants with varying hematocrit values. Normal term infants appropriate for gestational age preterms and small for gestational age infants were studied during the first week. Viscosity/hematocrits varied enormously both in different infants, but also between groups, i.e. terms, preterms and SGA. Marked variations occurred during the first week of life The normal viscosity values were found to be roughly twice as high as in adults, whereas the plasma viscosity was the same or rather lower The difference in viscosity in relation to adults seems to be due almost entirely to the higher hematocrit values. With late clamping of the cord infants (and especially small for gestational age infants) may have extremely high hematocrit and viscosity values.

KEY WORDS Newborn infant blood viscosity

Blood viscosity is affected by a number of factors such as shear rate hematocrit size and shape of erythrocytes abnormal hemoglobins temperature pH plasma proteins and plasma lipids (3 7 9 18 20 23) Among these factors the shear rate and hematocrit are of the greatest significance Few studies have been made of the conditions in the new born The present study was carried out in order to chart these hitherto relatively unknown conditions in greater detail to obtain a more reliable appraisal of the conditions during the first week of life and to ascertain the possible significance of pathological findings Preliminary results have been published earlier (4)

MATERIAL AND METHODS

A Pilot study comprising 65 newborn infants with varying hematocrit and covering both sick and healthy infants

B Normal term infants. Twenty infants appropriate for gestational age born by normal delivery with normal Apgar score partus in week 38–41 mean weight 3551 g, S D 481 g. Viscosity (whole blood and plasma) has been determinated in umbilical cord blood whole-blood viscosity at 1 3 and 5 days of age with a deviation of 7–8 hours Umbilical cord blood (whole blood) has been subjected to seven additional investigations To determine the variation and the error of the method an additional 10 infants (normal term) of mean weight 3 490 g (S D 315 g) have been examined on the third day of life duplicate samples being drawn simultaneously from two venipunctures. This group also constitutes a control group in relation to the former

C Eleven preterm infants with mean length of pregnancy 34 6 weeks, S D 1.2 weeks (range 33–36) and mean weight 2 ?45 g. S D 310 g were tested for whole-blood viscosity at 1 3 and 5 days of age as above

D Eleven small for gestational age (SGA) infants with mean length of pregnancy 39 1 weeks S D ? 0 weeks (range 35–41) and mean weight ? 375 g S D 258 g were tested for whole blood viscosity in analogy with group C The infants were outside −? sigma, according to Swedish standard curves for newborn infants (6).

E In 14 infants 1–5 days of age venous samples were drawn before and after 3 minutes of stasis

F From ?4 of the samples (A–B) chosen at random the correlation between viscosity at different shear rates and hematocrit (range 50–73) has been calculated

Hematocrit (%)	
Mean	S D
51.59 (n 77)	3 03

Hematocrit
(49 58<51 59<53 60)

Hematocrit %	
Mean	S D
63 09 (n 16)	6 33
6_ 13 (n=18)	3_3
57.96 (n=18)	4 86

Hematocrit
(59 74<63 09<66 64)
(60 6_<6_ 13<63 64)
(55 53<57 96<60 39)

variation is between individuals while there is very slight variations between samples The error of the method (according to the standard formula $\sqrt{d^2/2n}$) is for hematocrit 0.30 for viscosity at shear rate 23 sec^{-1} 0.51 46 sec^{-1} 0.34 115 sec^{-1} 0 14 and 230 sec^{-1} 0 05

The viscosities for erythrocyte concentrates are shown in Table 4 which also contains the corresponding values found by Litwin et al (13) for blood in adults

Table 5 shows the viscosities in 11 preterm infants with indication of mean value S D and 95% confidence interval

Table 6 shows the same for 11 SGA infants

Statistical analysis by means of the t-test (Table 7) shows a significant difference between SGA and preterms ($p<0\ 01$) on days 1 and 3 for both hematocrit and viscosity while between terms and preterms there is a difference of ($p<0\ 05$) on days 1 and 3 No significant difference between SGA groups was recorded on day 5 Nor was there any significant difference between terms and SGA infants Statistical analysis with paired t-test for the difference (Table 7) shows significant changes ($p<0\ 01$) both in the terms and in the SGA group between days 3 and 5 Mean hematocrit values before and after 3 minutes of venous stasis were 60 8 and 61.5% respectively a non-significant difference by the t-test

The correlation and regression coefficients between hematocrit and viscosity are shown in Table 8

DISCUSSION

Whereas the viscosity conditions in adults are fairly constant even if certain changes may be observed in states of illnesses and trauma (due to changes in hematocrit serum proteins plasma lipids and electrolytes) (3 4 17 18) these changes are nevertheless moderate

Table 4 *Erythrocyte concentrate viscosity values in cP*

Shear rate (sec^{-1})	5 75		11.5		_3		Hematocrit	
	Mean	S D	Mean	S D	Mean	S D	Mean	S.D
	133.0 (n=16)	41 3	119 6 (n=14)	35.3	9_ 3 (n=11)	26 1	96.6 (n=16)	1._
Litwin et al (13)	134._	21 9	110 6	18 6	85	7.5		

Table 1 *Values of viscosity (cP) at different shear rates for umbilical blood in normal new born infants*

Shear rate (sec^{-1})	23		46		115		230	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Whole blood	9.55 ($n=27$)	2.31	7.44 ($n=27$)	1.40	6.57 ($n=27$)	1.31	5.86 ($n=27$)	1.21
Plasma	1.72 ($n=19$)	0.27	1.64 ($n=19$)	0.19	1.57 ($n=20$)	0.12	1.56 ($n=20$)	0.10

95% confidence interval

Shear rate (sec^{-1})	23	46	115	230
Whole blood	(8.63<9.55<10.47)	(7.40<7.94<8.48)	(6.05<6.57<7.09)	(5.38<5.86<6.34)
Plasma	(1.59<1.72<1.85)	(1.55<1.64<1.72)	(1.51<1.57<1.63)	(1.51<1.56<1.61)

Table 2 *Whole blood viscosity (cP) during the first week in 20 normal new born infants*

Shear rate (sec^{-1})	23		46		115		230	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Day 1	17.45 ($n=16$)	3.26	13.99 ($n=16$)	2.6[illegible]	11.18 ($n=16$)	1.62	9.63 ($n=16$)	1.82
3	16.12 ($n=18$)	2.30	12.86 ($n=18$)	05	10.41 ($n=18$)	1.46	9.33 ($n=18$)	1.27
5	15.17 ($n=18$)	2.79	12.25 ($n=18$)	28	9.59 ($n=18$)	1.68	8.40 ($n=18$)	1.49

95% confidence interval

Shear rate (sec^{-1})	23	46	115	230
Day 1	(15.72<17.45<19.18)	(12.60<13.99<15.38)	(10.32<11.18<12.04)	(8.67<9.63<10.59)
3	(14.94<16.12<17.30)	(11.81<1[illegible].86<13.91)	(9.68<10.41<11.34)	(8.69<9.33<9.97)
5	(13.78<15.17<16.56)	(11.11<12.25<13.39)	(8.75<9.59<10.43)	(7.55<8.40<9.15)

tions within the pilot study at shear rate 23 and 230 sec^{-1} while the third shows the various viscosities at shear rates varying from 11.5 to 240 sec^{-1} for 8 infants The general appearance of the curves is very similar to that reported both for adults and children Viscosities both for whole blood and plasma (mean S D and 95% confidence interval) for umbilical cord blood are shown in Table 1 the blood viscosities at 1 3 and 5 days of age for the normal group in Table 2 (again with mean S D and 95% confidence interval) The loss of samples on the different days have been due to chance factors

The control group mean hematocrit was 61.20 S D 3.12 The *t* test shows no significant difference between the latter and the normal group Analysis of variance of the duplicate samples (hematocrit) and viscosity at shear rate 230 sec^{-1} for the control group shows (Table 3) that almost the whole of the

Table 3 *Analysis of variance*

Source of variation	df	SS	MS	F
Hematocrit				
Between	9	171.30	19.03	63.44
Within	10	3.00	0.30	
Total	19	174.30		
Viscosity at shear rate 23 sec				
Between	9	22.18	2.48	45.68
Within	10	0.54	0.05	
Total	19	22.82		

Hematocrit	
Mean	S D
58 00 (n 11)	4 78
55 68 (n 11)	4 46
56 70 (n=10)	3 48

Hematocrit
(55 06<58 00<60 94)
(52 68<55 68<58 68)
(54 1<56 70<59 19)

Hematocrit	
Mean	S D
65 82 (n=11)	5 1
64 59 (n=11)	5 58
60 85 (n 11)	5 78

Hematocrit
(62 37<65 82<69 32)
(60 84<64 59<68.34)
(56 72<60 85<64 98)

viscosity at some hematocrits (40–50%) has been in acceptable agreement with studies on adults (3 9 18) The viscosity of erythrocyte concentrates was also about the same as has been reported in adults (13)

The hematocrit values in this study were elevated for all groups The most common explanation of the variation in hematocrit between different groups of infants is the different procedures used for clamping of the cord (12, 15 16 22) In this material late clamping (3–5 minutes post partum) is standard and the present values are in close conformity with other studies on late clamped

Table 7 *Changes in viscosity and hematocrit during the first week*
(a) *Difference between the days*

	Term	Preterm (PT)	SGA
Day 1 / Day 3	NS	NS	NS
Day 3 / Day 5		NS	
Day 1 / Day 5		NS	

(b) *Difference between th group*

	SGA/PT	SGA/Term	Term/PT
Day 1		NS	
Day 3		NS	
Day 5	NS	NS	NS

** $p<0\ 01$ $p<0\ 05$ NS $p>0\ 05$

infants (15) In the present investigation the upper limit for the 95% confidence interval for the hematocrit at 1 day of age would appear to be 66 64 for normal term infants and 69 32 for SGA infants

In conjunction with high hematocrit values there have been reports of conditions showing signs of both peripheral and central circulatory insufficiency (2 5 11 24) and often the limit for normovolemic hemodilution has been given as 70 in venous hematocrit corresponding to a blood viscosity of roughly 3 times that in the normal adult With late clamping of the cord it is easy to come in

Table 8 *Correlation and regression coefficients between hematocrit and blood viscosity at different shear rates in newborn infants*

Hematocrit (x) Viscosity (y) (n=24)
Hct 50–73

Viscosity at shear rate (sec)	Correlation	Regression
11.5 (n=11)	0.945	0 781
23	0.947	0.571
44	0 943	0 474
115	0.918	0 348
230	0 909	0.281

Hematocrit	
Mean	S D
58.00	4.38
(n 11)	
55.68	4.46
(n 11)	
56.70	3.48
(n 10)	

Hematocrit
(55.06<58.00<60.94)
(52.68<55.68<58.68)
(54.21<56.70<59.19)

Hematocrit	
Mean	S D
65.82	5.1
(n 11)	
64.59	5.98
(n 11)	
60.85	5.78
(n 11)	

Hematocrit
(62.32<65.82<69.32)
(60.84<64.59<68.34)
(56.7?<60.85<64.90)

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(b) *Difference between the group*

	SGA/PT	SGA/Term	Term/PT
Day 1		NS	
Day 3		NS	
Day 5	NS	NS	NS

$p<0.01$ $p<0.05$ NS $p>0.05$

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46	0 943	0 474
115	0.918	0 348
230	0 909	0 281

the vicinity of these values especially in SGA infants None of the neonates in this study however had any signs of circulatory impairment (groups B–F)

The shape of the curves in Figs 1–3 is very similar to the exponential curves described for adult blood If the relation between viscosity and hematocrit is expressed as a linear function Litwin et al (13) calculated the correlation coefficient to be 0 8–0 9 with hematocrit variation 36–52% For most newborn infants the correlation is still better with correlation coefficients more than 0 90 Therefore it should be possible to use venous hematocrit instead of viscosity measurements for simple clinical purposes

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BLOOD VISCOSITY AND PERIPHERAL CIRCULATION IN NEWBORN INFANTS

A Study on Resting Flow

GUDMUND BERGQVIST and ROLF ZETTERSTRÖM

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ABSTRACT Bergqvist, G and Zetterström, R. (Department of Paediatrics, Karolinska Institutet, St Goran's Hospital for Children, Stockholm, Sweden). Blood viscosity and peripheral circulation in newborn infants. A study on resting flow Acta Paediat Scand, 63:865, 1974.—Whole blood iscosity hematocrit and peripheral resting flow have been measured in 18 one to three days old newborn infants. There was no correlation between viscosity and blood flow or between viscosity and peripheral resistance. In seven infants hervolemic hemodilution was performed. This procedure did not alter the resting blood flow

KEY WORDS: Newborn infants, blood viscosity resting peripheral blood flow

h increasing whole blood viscosity there ı decrease of peripheral flow as demon- ıted in animal experiments on hindleg cimens or isolated rabbit ear (13 15 22) ce however there may be differences ween viscosities *in vivo* and *in vitro* the blem is much more complex than a simple ıtion between peripheral circulation and od viscosity (7 23)

n newborn infants the hematocrit is high whole blood viscosity considerably ele- ed compared to adults (3 17) Peripheral ulation is characterized by high peripheral scle) flow low resistance and excellent omotor activity (2, 5 6) Since some in- ts with elevated hematocrit have central peripheral circulatory failure (1 10 18 it has been considered to be of clinical rest to study the influence of blood cosity on flow in newborn infants

MATERIAL AND METHODS

teen newborn infants 1 to 3 days old with normal ncreased hematocrits and with birthweights varying between 1950 and 4580 g and gestational age between 36 and 43 weeks have been studied. Pregnancies and deliveries were normal. Five of the infants were small for gestational age according to Swedish standard curves for newborn infants (8). Apgar scores were 8 to 10 at 3 minutes. The postnatal course was uneventful

Peripheral circulation was studied by venous occlusive plethysmography in the foot and calf as described by Celander and co-workers (? 5 6). The technical aspects of this method have been thoroughly commented upon by Sigdell (21)

All the measurements were done in a standardized manner The babies had been fed immediately before the study and were sleeping They were placed in an incubator at a temperature of 32 to 33°C The temperature in the plethysmograph was 34 to 35°C with fluctuation between 32 and 37°C in a few cases. Peripheral circulation has been found to be stable under these environmental conditions (?). The measurements were done during a period of 30 to 60 minutes

The flow was calculated as the mean of 3 to 5 measurements which were performed within 5 minutes. Venous occlusive pressure was 40 mmHg. Systolic pressure was determined plethysmographically (6)

Blood viscosity and hematocrit were determined as earlier described (3) All blood flow measurements were performed 1 hour after blood had been drawn to avoid the influence of changed vasomotor activity

In 7 of the infants flow was measured before during and after an isovolemic hemodilution Blood (20 to 30 ml) was then substituted by a solution consisting of one part of 20% serum albumin solution and two

Table 1 *Blood flow hematocrit blood viscosity blood pressure heart rate and periph. resistance in 18 newborn infants*

	Flow ml/min/ /100 ml tissue	Hematocrit	Viscosity (cP) shear rates (sec⁻¹) 23	46	115	230	Blood pressure (mmHg)	Heart (rate/min)	PRU^{0}_{100}	PRU^{20}_{100}
Mean	7.9	64.6	17.1	14.1	10.8	9.5	65.5	133.9	9.8	6.7
S D	3.4	4.6	3.0	2.3	1.8	1.7	6.5	10.6	4.6	3.1
S E M	0.8	1.1	0.8	0.6	0.5	0.4	1.5	2.5	1.1	0.7
Range	2.5–15.6	56–71	10.9–20.1	9.4–16.3	6.9–1 8	6.0–11.4	55–80	116–155	4.2–25	3–17

Peripheral resistance is given with capillary pressures assumed to be 0 and 20 mmHg written as PRU^{0}_{100} and PRU^{20}_{100}

parts of a solution with a glucose concentration of 5.5% and one part of a solution of sodium chloride concentration of 0.9%

RESULTS

Mean values standard deviation (S D) standard error of mean (S E M) and range for hematocrit blood viscosity at different values blood flow blood pressure heart rate and calculated peripheral resistance are given in Table 1 Peripheral resistance is given with capillary pressures assumed to be 0 and 20 mm written as PRU^{0}_{100} and PRU^{20}_{100} The following standard formula has been used

$$PRU = \frac{\text{Blood pressure} - \text{capillary pressure}}{\text{flow}} \times \frac{\text{mmHg}}{\text{ml/100 ml tissue/min}}$$

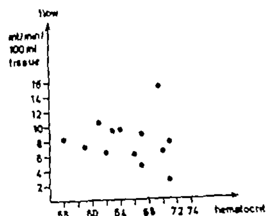


Fig 1 Relation between hematocrit and resting blood flow in 18 newborn infants.

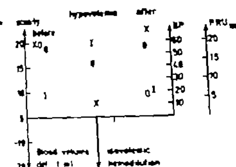
The relation between hematocrit and blood flow is shown in Fig. 1

The correlation between blood viscosity at different shear rates and hematocrit was found to be highly significant ($p<0.001$) There was no correlation between hematocrit/viscosity and flow heart rate peripheral resistance or blood pressure Finally the correlation between peripheral resistance and flow was highly significant ($p<0.001$ $r=-0.735$) and between blood pressure and flow significant ($p<0.01$ $r=0.608$) Between heart rate and flow the correlation was almost significant ($p<0.05$ $r=-0.43$) There was no difference between infants who were small for gestational age and those who were appropriate for gestational age

The results of isovolemic hemodilution are shown in Table 2 The correlation between flow change was not significant Fig. 2 demonstrates the changes of flow viscosity peripheral resistance and blood pressure in one of the babies subjected to hemodilution. The reduction of the total blood volume was followed by an elevation of peripheral resistance and a reduction of flow There was no effect of the reduction of the viscosity

COMMENTS

Peripheral circulation in newborn infants has been extensively studied by Celander (5) Our data for resting peripheral flow and re



. 2. Changes in blood viscosity (cP at shear rate 23 ⁻¹=•), blood pressure (mmHg=0) peripheral re ance (PRU=I), and blood flow (ml/min/100 ml tis =X) in a newborn infant before and 30 min after odilution and hypovolemia.

ance are in close agreement with those orted by Celander in normal newborns the mean hematocrit was elevated (17)

t is a well-known fact that newborn in- ts with high hematocrit and elevated blood cosity may develop symptoms suggestive circulatory failure (1 10 18 24) Since results of various animal experiments e demonstrated that peripheral flow may influenced by viscosity (13 15 19 20 , we have examined if there is in the born infant any correlation between rest blood flow as measured in the calf and t and viscosity of the blood. However n our results it is evident that the resting d flow in the calf and foot of the new- n is not under influence of variations in hematocrit when it does not exceed a e of about 70%

t can only be speculated upon why the ng blood flow in the calf and foot of born infant is not correlated to the he ocrit if the value is below 70% In hu- adults Gelin & Thorén using venous lusive plethysmography have not found effect on resting flow when there is no regation tendency in the blood (11) In born infants the aggregation tendency is as demonstrated by the fact that the throcytes have a high suspension stability low sedimentation rate By the use of an tope technique Lewis et al have found that muscle flow is unaffected by hemodilu tion (14) It also has to be considered that there are marked differences of *in vivo* and *in vitro* viscosity (7) With diminishing diameter the blood viscosity becomes more similar to plasma as found from studies of the blood in glass capillaries (9 12) Another factor influencing the relation between viscosity and flow is blood volume Hypervolemia which is known to improve flow (16 20) has been demonstrated to occur in new born infants with high hematocrit values (4)

The discrepancy between our results and those of animal experiments (13 15 19 20 22) may also be that maximal flow has been measured in the animal experiments since the vasomotor activity of the capillary re gion was blocked whereas there is an enor mous reserve flow capacity in muscles (7) i e the main tissue studied by us

Thus it may very well happen that resting flow is normal although maximal flow is impaired

There is reason to stress that many fac tors have an influence on the peripheral cir culation Blood volume cardiac output blood pressure and vasomotor activity have a much more pronounced effect than viscosity but in a low flow state the effect of viscosity may be crucial

ACKNOWLEDGEMENTS

Our thanks are due to Dr O Celander for valuable instructions.

Table 2. *Changes in viscosity (cP) at shear rate 23 sec⁻¹ blood pressure (mmHg) blood flow (ml/100 ml tissue/min) and peripheral resistance (PRU-units) in 7 newborn infants following isovolemic hemodilution*

Parameter	Mean	S D	Median	Significance
Viscosity	−5.5	3.0	−5 0	$p<0.01$
Blood pressure	4.3	7 7	2.0	ns
Blood flow	−0.4	1 4	−0.8	ns
Peripheral resistance	4 4	6.9	0 9	ns

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THE EFFECT OF ACETYLSALICYLIC ACID ON PLATELET FUNCTION IN CYANOTIC CONGENITAL HEART DISEASE

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ABSTRACT Goldschmidt, B., Sørland S. J and Bjørnstad P. G. (Second Department of Paediatrics, Semmelweis University Medical School, Budapest, Hungary; Institute for Thrombosis Research and Department of Paediatrics, Rikshospitalet, Oslo, Norway). The effect of acetylsalicylic acid on platelet functions in cyanotic congenital heart disease. Acta Paediatr Scand, 63:869 1974.—Children with cyanotic congenital heart disease (CCHD) are susceptible to thromboembolic complications. The pharmacologic inhibition of platelet function may be efficacious in the prophylaxis of thrombosis. The authors have studied the platelet count, Ivy and Duke bleeding time, platelet adhesiveness *in vivo* and *in vitro* thrombelastogram, spontaneous platelet aggregability and platelet aggregation induced by collagen, ADP and adrenaline in children with CCHD before and after ingestion of acetylsalicylic acid (ASA) or placebo. Before treatment, some of the patients had thrombocytopenia, prolonged Ivy bleeding time, increased platelet adhesiveness in vivo and in vitro and increased spontaneous platelet aggregation. In a placebo group, there was no significant change in the pre- and post-treatment values of the investigated indices. After ASA treatment there was no significant change in the platelet counts, Duke bleeding time and thrombelastogram There was a significant prolongation of Ivy bleeding time, decrease of *in vivo* and *in vitro* platelet adhesiveness and normalization of the spontaneous platelet aggregation. ASA inhibited the secondary wave of aggregation induced by small doses of ADP or adrenaline and decreased aggregation induced by collagen. These data suggest that ASA may be suitable for prevention of thromboembolic complications in CCHD.

KEY WORDS: Cyanotic congenital heart disease, platelet functions, acetylsalicylic acid

Children with cyanotic congenital heart disease (CCHD) and secondary polycythemia are susceptible to arterial thromboembolism (6 10). It is often also the immediate reason for death in some types of thrombotic episodes (22) On the other hand alongside the thrombotic tendency there is a bleeding susceptibility in these patients (8) The origin of the abnormal coagulation in the majority of cases is the existing chronic form of disseminated intravascular coagulation (DIC) and consumption coagulopathy (10 17) The thrombotic tendency is probably a sequence 1) of rheological factors (secondary polycythemia, raised whole-blood viscosity reduced blood flow) 2) of vessel wall damages secondary to a hypoxaemia, and 3) of the increased adhesiveness and aggregability of platelets (9) It is believed that adhesion of platelets onto the damaged vessel wall of subendothelial connective tissue and the subsequent events that lead to platelet aggregation and release reaction, is a central point in the pathogenesis of DIC

Currently heparin is the treatment of choice for DIC and consumption coagulopathy (10) However there arises the de

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Table 1. *Results of placebo and treatment with acetylsalicylic acid on platelet function in CCHD*

Test	Normal values	Patients with CCHD								
		Pre-treatment values					Post-treatment differences			
		Placebo group		ASA group			Placebo group		ASA group	
		No. of cases	Mean (±S.D.)	No. of cases	Mean (±S.D.)	Significance p	Mean (±S.D.)	Significance p	Mean (±S.D.)	Significance p
Platelet count, $10^3/mm^3$	150–350	1[illegible]	178 (7[illegible])	3	163 (73)	>0.05	−6 (11)	>0.05	+11 (11)	>0.05
Bleeding time										
Ivy sec	40–480	1	51 (65)	22	504 (62)	>0.05	+26 (31)	>0.05	+195 (190)	<0.001
Duke sec	60–200	1	160 (57)	22	168 (57)	>0.05	−8 (25)	>0.05	+20 (30)	>0.05
Adhesiveness										
In vivo, %	20–50	12	60.2 (7.3)	22	56.4 (7.1)	>0.05	−2.7 (6.1)	>0.05	−8.4 (15)	<0.05
In vitro, %	20–50	1	58.[illegible] (7.5)	22	57.7 (7.3)	>0.05	−4.[illegible] (8.3)	>0.05	−16.[illegible] (1[illegible]5)	<0.001
Thrombelastogram										
r, min	6–11	10	9.70 (1.[illegible])	13	8.70 (1.[illegible])	>0.05	+0.18 (0.8)	>0.05	+0.03 (0.8)	>0.05
k, min	3–6	10	3.30 (0.8)	13	3.26 (0.8)	>0.05	+0.05 (0.4)	>0.05	−0.13 (0.6)	>0.05
ma, mm	46–60	10	56.9 (4.6)	15	54.6 (4.4)	>0.05	−0.9 (3.0)	>0.05	−0.9 (3.0)	>0.05

The results of the placebo and ASA study are summarized in Tables 1, 2 and 3.

Placebo study

There was no significant change in the pre and post-treatment values of the investigated indices.

ASA study

There was no significant difference between the pre and post-treatment values of platelet counts, Duke bleeding time and data of TEG (*r*, *k* and *ma* values).

The bleeding time determined by Ivy showed a significant prolongation in all subjects after ASA (Fig. 1).

Table 2. *Results of platelet aggregation tests (PAT) in children with cyanotic congenital heart disease before and after placebo and acetylsalicylic acid (ASA)*

Drug	No. of cases	Stages of PAT					Significance, p		
		1		3	4	5			
Placebo									
Before	1	4	3	3	2	–			
After	1	5	2	3		–	>0.05	>0.05	
ASA									<0.01
Before	22	7	4	6	5	–			
After	[illegible]2	14	8	–	–	–	<0.05		

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		No of cases	Mean (±S D)	No of cases	Mean (±S.D)	Significance *p*	Mean (±S.D)	Significance *p*	Mean (±S D.)	Significance *p*
Platelet count, $10^3/mm^3$	150–390	1	178 (72)	3	163 (73)	>0 05	−6 (11)	>0 05	+11 (11)	>0.05
Bleeding time										
Ivy sec	40–400	1	51 (65)	22	504 (6_)	>0 05	+26 (31)	>0 05	+195 (190)	<0 001
Duke, sec	60–200	1	160 (57)	22	168 (57)	>0.05	−8 (25)	>0.05	+70 (30)	>0 05
Adhesiveness										
In vivo, %	20–90	12	60_ (7.3)	_2	56.4 (7 1)	>0 05	−_7 (6 1)	>0.05	−8.4 (15)	<0 05
In vitro, %	70–90	1	58.2 (7.5)	22	57 7 (7 3)	>0 05	−4 (8 3)	>0 05	−16_ (1 5)	<0 001
Thrombelastogram										
r mm	6–11	10	9 20 (1 2)	15	8 70 (1)	>0 05	+0 18 (0 8)	>0 05	+0.03 (0.8)	>0 05
k, mm	3–6	10	5.50 (0 8)	15	3 26 (0 8)	>0 05	+0.05 (0 4)	>0 05	−0.13 (0 6)	>0 05
ma mm	46–60	10	56.9 (4 6)	15	54 6 (4 4)	>0.05	−0.9 (3 0)	>0 05	−0.9 (3 0)	>0 05

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Placebo									
Before	12	4	3	3		–	>0 05	>0 05	
After	1_	5	2	3		–			
ASA									<0 01
Before	22	7	4	6	5	–	<0.05		
After	22	14	8	–	–	–			

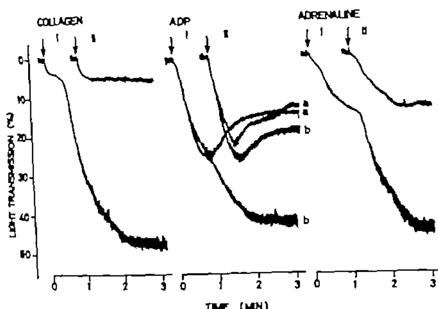


Fig. 2. Platelet aggregation induced by collagen (final concentration 4.2 µg/ml), ADP (final concentration 0.6 µg/ml (a) and 1.2 µg/ml (b)) and adrenaline (final concentration 3.64 µM) in PRP of children with CCHD before (I) and after (II) ASA treatment. Representative experiment.

tion showed a characteristic change after ASA ingestion (Fig. 2). The values of maximum aggregation decreased in all of the cases with collagen and both concentrations of ADP. The secondary wave of aggregation did not occur with adrenaline. The maximum aggregation showed a highly significant decrease on collagen and adrenaline induced aggregation and a significant decrease on ADP induced aggregation (Table 3).

DISCUSSION

It is only in recent years the applicability of drugs which inhibit platelet function with the aim of thrombosis prophylaxis has been examined. On the basis of comparative investigations acetylsalicylic acid (ASA) is most suitable (7). Low doses of ASA have been shown to increase the bleeding time in some normal subjects and have also been shown to decrease the adhesivity of platelets on foreign surfaces both in vivo and in vitro (14). ASA has been shown to decrease platelet aggregation with connective tissue and with low concentrations of thrombin and to inhibit the secondary wave of aggregation with ADP and adrenaline (20). These effects are thought to be due to inhibition of the release reaction and it has been suggested that its effect on hemostasis may be related to the acetylation reaction (1). The inhibitory effect after a single oral dose of ASA may be detected after 4–7 days. The inhibitory effect of ASA on platelet aggregation suggests its possible use as an antithrombotic agent and studies indicate that ASA ingestion prevents some types of experimentally induced arterial thrombosis in dogs (4), in mice (19) and rabbits (16). In man doses of 1.5 g per day were found to reduce thromboembolic complications post-operatively (15, 21) and after transplantation of artificial heart valves (11) significantly. ASA was observed to reduce the deposition of thrombi in an extracorporeal shunt (5). In contrast to this, other authors did not find favourable effects of ASA in the prevention of thromboembolism (18).

We have studied the suppression effect of ASA on the platelet function *in vivo* and *in vitro* in children suffering from CCHD. In a majority of the cases ASA reduced the

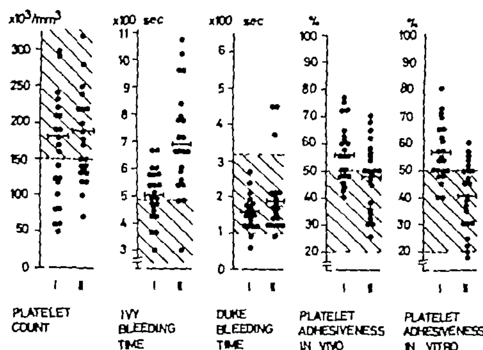


Fig 1 The platelet count Ivy bleeding time Duke bleeding time platelet adhesiveness *in vivo* and *in vitro* in children with CCHD before (I) and after (II) administration of ASA Shaded areas represent the normal ranges. Horizontal bars represent means.

There was a significant decrease in platelet adhesiveness *in vivo* and a highly significant decrease of adhesiveness *in vitro* The mean post ASA values were in the normal range However in spite of the ASA treatment in a few of the cases the adhesiveness remained in the pathologically increased range (Fig. 1)

The spontaneous aggregation of platelets before ASA treatment was increased in 11 out of 22 cases (Table 2) It was normal after treatment in all cases The change was statistically significant

The shape of the curve of the collagen ADP and adrenaline induced platelet aggrega

Table 3 *Results of placebo and treatment with acetylsalicylic acid on platelet aggregation induced by collagen ADP and adrenaline in children with CCHD*

Aggregating agents (final concentration)	Platelet aggregation[a]								
	Pre-treatment values					Post-treatment differences			
	Placebo group		ASA group			Placebo group		ASA group	
	No of cases	Mean (±S D) %	No of cases	Mean (±S D) %	Significance p	Mean (±S D) %	Significance p	Mean (±S D) %	Significance p
Collagen (4 0 μg/ml)	7	53 0 (4 4)	14	51 0 (4 6)	>0 05	−0 8 (2 1)	>0.05	−46 (3 1)	<0.001
ADP (0.6 μg/ml)	7	24 6 (3 0)	14	25 0 (3 1)	>0.05	−0 5 (3 3)	>0 05	−2.4 (? 3)	<0 01
ADP (1 2 μg/ml)	7	49 2 (3 9)	14	48 6 (4 0)	>0.05	+0 2 (? 8)	>0 05	−22.0 (2.6)	<0.05
Adrenaline (3 64 μM)	7	51 0 (4 5)	14	49 4 (4.5)	>0 05	+0 6 (3 0)	>0.05	−33 (3.2)	<0 001

[a] Maximal change in light transmission (%)

Acta Pædiatr Scand 63 875–877 1974

CLINICAL MANIFESTATIONS OF INFECTION WITH YERSINIA ENTEROCOLITICA IN CHILDREN

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ABSTRACT Bergstrand C. G. and Winblad, S. (Department of Paediatrics and Clinical Bacteriology Malmö General Hospital, Malmö, Sweden). Clinical manifestations of infection with Yersinia enterocolitica in children Acta Paediatr Scand, 63: 875 1974.—The case records of 31 hospitalized children infected with *Yersinia enterocolitica* during the period January 1967 to September 1973 were retrospectively analysed. Most of the children were under 4 years of age and all had been admitted with a diagnosis of gastroenteritis. The clinical picture was essentially the same as in other types of acute or subacute gastroenteritis and specific signs permitting a clinical diagnosis of Yersiniosis were not observed. The symptoms were of mild to moderate degree, and all patients had an uneventful recovery Erythema nodosum and arthritis were not observed and more severe abdominal pains, usually considered typical for older children and adults with Yersiniosis, registered in only 4 of the 31 patients.

KEY WORDS: *Yersinia enterocolitica*, Yersiniosis, gastroenteritis, children

Certain O-serotypes (O3 O8 O9) of *Yersinia enterocolitica* are pathogenic to man causing (inter alia) gastroenteritis acute terminal ileitis and mesenteric lymphadenitis (7 12–15) Association with arthritis and erythema nodosum has also been demonstrated (1 13) Yersiniosis enterocolitica has been shown to be a fairly frequent cause of enteritis in small children in Sweden (2) Similar findings have also been reported in Belgium (11) Finland (1) and Canada (3) The clinical patterns of *Yersiniosis enterocolitica* in children have mostly been described in reports of small numbers of patients A more extensive study has recently been published by Delorme et al (3) The aim of the present investigation was to analyse the symptomatology of a number of children with a confirmed infection of *Yersinia enterocolitica* and with a clinical diagnosis of gastroente...

MATERIAL AND METHODS

During the period January 1967 through September 1973 31 infants and children who were admitted to the Department of Paediatrics at Malmö General Hospital were found to be infected with *Yersinia enterocolitica* as shown through isolation of the microorganism in stool specimens. All of them were referred with a diagnosis of gastroenteritis. Since stool cultures were not routinely done for all patients admitted with diarrhoea, the clinical material should be considered selected Cases of Yersiniosis with pseudoappendicitis, i with symptoms resembling acute appendicitis but found at operation to have a normal appendix with or without mesenteric lymphadenitis, were treated i the surgical department and not included in the material

The pattern of symptoms, duration of the illness and other relevant data were retrospectively analysed based on the case records.

The isolation of *Yersinia enterocolitica* in the faeces was the main diagnostic criterion in all patients, and cultures were prepared as described by Nilehn (6). In addition the diagnosis was confirmed by positive serological findings (2) in 10 of the 12 patients so examined The titre of agglutinating antibodies ranged from 1/80 to 1/5 120. Isolated strains belonged to serotype O3

pathologically increased platelet adhesiveness both *in vivo* and *in vitro* to the normal range It inhibited the pathologically increased spontaneous platelet aggregation It inhibited the secondary wave of aggregation induced by small doses of ADP and adrenaline and decreased aggregation with collagen On the basis of our earlier work (8 9 10) we feel that platelets play an important role in thrombogenesis in the cyanotic patients The results of present study suggest that application of ASA may be successful in the prevention of thromboembolic complications also in these groups of patients

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compatible with Yersiniosis in parents and siblings and the mostly negative bacteriological and serological findings in the families examined suggest a rather low degree of contagiousness.

The clinical picture varies among adults infected with *Yersinia enterocolitica* (1 2 7 11 13 15). Besides symptoms in the gastrointestinal tract erythema nodosum and arthritis have been observed In children the latter symptoms must be rare and were not reported among the present patients The most impressive signs in childhood are acute and subacute relatively mild gastroenteritis Judging from this and previous investigations (3 11) pathognomonic symptoms do not allow a specific clinical diagnosis A specific diagnosis of *Yersinia enterocolitica* thus must be based upon bacteriological and serological findings. Abdominal pains resembling those of acute appendicitis have been considered characteristic of infections with *Yersinia enterocolitica* in older children and adults (1 2, 11). Severe pains were apparently not common among the younger patients studied here this symptom was specifically register ed for only 4 of the 31 cases It is possible that children with severe abdominal pains were referred to the surgical department and that this symptom is thus somewhat more frequent than observed in the present material It may be concluded from this and other reports (3 11) that the illness associated with *Yersinia enterocolitica* in children at least in the younger age groups usually is a self limited and relatively mild infection without serious complications Exceptions to this rule have however recently been reported Gutman et al (5) described the fatal outcome of a septicemia caused by *Yersinia enterocolitica* in a 19-month-old boy The isolated strain in this case however belonged to serotype O8 Development of chronic forms of enteritis has also been observed (3) Treatment with antibiotics does not seem necessary but may be considered when the infection runs a prolonged course.

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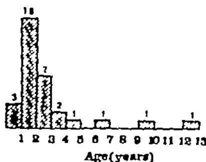


Fig 1 Age distribution

RESULTS

The age distribution of the patients is illustrated by Fig 1 The majority were under 4 years of age and only 6 of 31 were older than 3 years The material consisted of 12 boys and 19 girls Most of the patients (80%) were admitted during the months June through December The clinical manifestations were those of acute or subacute gastroenteritis (Fig 2) All of the patients had diarrhoea of varying intensity and duration but none required intravenous administration of fluid and their general condition was usually not impaired Blood in the stools was observed in 2 patients and a conspicuous amount of mucous in the stools was found in one patient The duration of diarrhoea prior to admission ranged from 1 day to 3 weeks A history of diarrhoea for more than 10 days was exceptional however Vomiting as a pronounced symptom was reported for 9 children but abdominal pain was reported for only 4 Fever had been registered in about half of the patients The average durations of the illness was about 9 days with a range from 4 days to 4 weeks

The families were not systematically examined for evidence of Yersiniosis In 5 cases other members of the family were reported to have been ill with signs consistent with a diagnosis of Yersiniosis In 6 families other

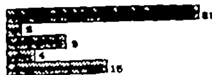


Fig 2 Clinical manifestations.

members than the patients were examined bacteriologically and/or serologically but the results were positive only in one family

Antibacterial treatment was given to 9 patients 5 receiving tetracyclines and 4 sulfonamides All 31 patients made an uneventful recovery and those not treated with antibacterial agents did not differ from those receiving such drugs with regard to the clinical course

DISCUSSION

Since stool cultures were not made in all patients admitted with gastroenteritis during the study period definite conclusions cannot be drawn concerning the general incidence of Yersinia infections This etiology of gastroenteritis may very well be more common than indicated by the 31 cases in the present investigation Infection with *Yersinia enterocolitica* may be associated with very mild symptoms of gastroenteritus and in such cases bacteriological examination may quite understandably have been considered unnecessary

Most of the patients belonged to the youngest age groups a finding which is in fairly good accord with the observations made by Delorme et al (3) The monthly incidence of infections with *Yersinia enterocolitica* found in the present study is in agreement with previous investigations made by Arvastson et al (2) and Vandepitte et al (11) and points to a higher incidence during the latter half of the year Delorme et al (3) found on the other hand maxima in March July and November

The source of infection with *Yersinia enterocolitica* is not yet known but an alimentary origin seems probable Hospital outbreaks and family and inter familial outbreaks (mostly serotype O3) have been reported (1 4 5 8–10) suggesting additional transmission through personal contact In the present study the frequency of secondary cases within the families was not systematically investigated but the low incidence of symptoms

hematocrit, i.e. above 70% and finally to what degree changes in hematocrit will affect renal function. Special attention has been paid to the changes in renal function which occur when an abnormally high hematocrit is reduced to values which can be considered normal for newborn infants (12) by the use of isovolemic hemodilution.

MATERIAL AND METHODS

Ten newborn infants with a high hematocrit syndrome have been included in the study. Eight of the infants were fullterm, two were born after 36 and 37 weeks of gestation, respectively. The weights of the infants were appropriate for gestational age (11). All babies had a normal delivery. Most of them were born in maternity hospital where late clamping of the umbilical cord is practised. During the first day after birth the infants exhibited clinical signs of polycythemia, i.e. had a "plethoric" face and had a venous hematocrit above 70%. Once the diagnosis of abnormally high hematocrit was established the infants were transferred to a neonatal ward here they were studied and handled according to the following protocol

1. Hematocrit and viscosity

Simultaneous determinations of venous hematocrit and viscosity were performed in 6 of the infants before and after a standardized isovolemic hemodilution. In the remaining 4 only venous hematocrit was determined. To establish the correlation between hematocrit and viscosity data were also included from 8 fullterm, newborn infants with abnormally high hematocrit in whom a similar hemodilution had been performed without determinations of renal function. To establish the relationship between hematocrit and viscosity at different shear rates additional data from previous study of newborn infants (6) have also been included.

2. Renal function studies

The renal response to an oral sodium chloride load and/or the glomerular filtration rate (GFR) has been determined. The infants received 0.1 g sodium chloride/kg body weight corresponding to 2 mEq Na per kg body weight, during standardized high fluid intake. Urine was collected at spontaneous voidings during 5 hours. GFR was determined by the use of single injection technique of inulin details have been reported in an earlier study (3).

3. Hemodilution

When renal function had been studied or the hematocrit and viscosity had been determined, an isovolemic hemodilution was performed through catheter inserted into the umbilical vein. The aim was to lower the hematocrit to about 60%. For the calculation of the amount of blood to be replaced the following formula was used (7):

$$\frac{BW \times BV\,(Hct_1 - Hct_2)}{Hct}$$

where

BW = body weight in kg
BV = blood volume (8.5% of BW has here been used)
Hct = actual hematocrit
Hct = desired hematocrit

The dilution was made under normothermic conditions with a solution consisting of 1 part of 20% albumin parts of 5.5% glucose and 1 part of 0.9% sodium chloride. Osmolality, sodium and albumin concentrations of the solution were approximately the same as in blood.

One to 14 hours after the dilution the renal function tests were repeated.

The response to the oral sodium load and the GFR was studied in 5 infants. In 3 of them only an oral salt load was given. In another only GFR was determined before and after hemodilution. In one of the infants the hematocrit decreased spontaneously after high fluid intake consequently no isovolemic hemodilution was performed.

Serum sodium, serum albumin and blood osmolality were also studied before and after hemodilution. Blood pressure was measured in 5 infants before and after hemodilution.

Analytical methods

The sodium concentration in serum and urine was analysed by flame photometer (Eppendorf). Inulin in blood was determined according to Heyrovsky (13). Osmolality in blood and urine was determined cryoscopically with the aid of a Knauer osmometer. Serum albumin was determined refractrometrically. Hematocrit was estimated in glass capillaries which were centrifuged at 11 000 rpm for 6 minutes. Determinations of whole blood viscosity were carried out with Brookfield's LVT viscometer at shear rates of 23, 46, 115, 230 sec. Details of the viscosity determinations in newborn infants have been reported earlier (6). Paired *t*-test has been used in statistical analyses.

RESULTS

Following isovolemic hemodilution the hematocrit was lowered from about 70 to 60% with individual variations as is shown in Fig. 1. The changes in viscosity parallel the changes in hematocrit. The differences of the mean values of the hematocrit and viscosity before and after the exchange transfusion are highly significant ($p<0.001$). A lowering of hematocrit of about 10% caused

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RENAL FUNCTION IN NEWBORN INFANTS WITH HIGH HEMATOCRIT VALUES BEFORE AND AFTER ISOVOLEMIC HAEMODILUTION

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ABSTRACT Aperia A Bergqvist G., Broberger O Thodenius, K and Zetterström R. (Department of Paediatrics, St Göran's Children s Hospital Karolinska Institutet Stockholm Sweden). Renal function in newborn infants with high hematocrit values before and after isovolemic hemodilution. Acta Paediatr Scand 63 878 1974.—To evaluate the effect of high hematocrit (secondary polycythemia) on renal function in newborn babies, 10 infants with values above 70% have been studied before and after an isovolemic hemodilution By replacing blood with a solution containing albumin glucose and sodium chloride the hematocrit was reduced to about 60% Hematocrit, blood viscosity glomerular filtration rate (single injection technique) and the renal response to an oral sodium and fluid load were determined before and after hemodilution. The fall in hematocrit was accompanied by a concomitant fall in blood viscosity All parameters of renal function which were studied were low before hemodilution but improved after this procedure Water excretion increased out of proportion to glomerular filtration rate. It is suggested that the reduction in renal function as seen in newborn infants with high hematocrit are secondary to an impairment of glomerular plasma flow which in turn is the consequence of high viscosity The depression of renal function as seen in polycythemic newborn infants may cause marked changes in the handling of certain drugs.

KEY WORDS: Newborn hematocrit, secondary polycythemia, renal function glomerular filtration rate viscosity hemodilution

It is generally agreed that renal function in newborn mammals is low for the size of the organism and for the weight of the kidney (9) This has been attributed to immaturity in structure (18) as well as to lack of induction from appropriate excretory need (10 16) It should however also be recognized that extrarenal factors known to influence renal function such as arterial blood pressure and hematocrit are different in the newborn using adult standards

Secondary polycythemia is physiological in the newborn infant The hematocrit generally ranges between 55 and 65% (6 12) When the hematocrit increases above 50% its effect on blood viscosity and thus resistance to flow will be pronounced (24) In the renal vasculature the blood will be further concentrated by the filtering process accentuating viscosity changes induced by polycythemia

The aim of the present study has been to examine whether the polycythemia of the newborn contributes to the low renal function at birth whether renal function is critically reduced in infants with abnormally high

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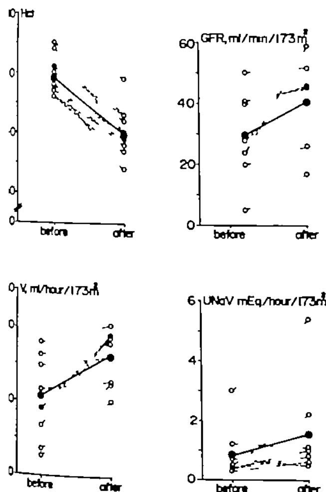


Fig. 3. The effect of hemodilution on the following parameters: hematocrit, glomerular filtration rate, urinary sodium excretion, diuresis. O=individual values, ●=mean values.

The exponential relationship between he tocrit and viscosity is well established en there is an increase of the hematocrit m 20 to 40% the effect on the viscosity ll shear rates is very small whereas there a rapid increase of viscosity at each unit t of the hematocrit when the hematocrit ceeds 50% (24). This fact was confirmed newborns in this laboratory with shear es ranging from 23 to 230 sec (6). The ar rates at which viscosity was studied y at least correspond to the blood flow ditions in the renal artery (23). It is pos le that even lower shear rates are true for blood flow in the pre and postglomerular erioles. However, it has been demonstrated that, if anything, the low shear rates will accentuate the effect of hematocrit on viscosity (20). Thus, it is apparent that changes in hematocrit between 60 and 70% may have important consequences for viscosity of the flow and thus for the resistance to flow.

Renal vasculature is unique in that it consists of two series-coupled arterioles, the pre and postglomerular arterioles, with the glomerular capillary network between them. In the glomerular capillaries the blood becomes concentrated by the filtering process. Thus, the hematocrit of the blood in the postglomerular arterioles will always be higher than in arterioles in other parts of the

a decrease in blood viscosity of about 30%. The relationship between hematocrit and viscosity at different shear rates is shown in Fig 2.

Fig 3 depicts the changes in renal function caused by hemodilution. When the hematocrit was reduced there was an almost significant increase in GFR ($p<0.05$). The mean value before dilution had been performed was 29.7±13.3 ml/min/1.73 m² which is at the lower normal range in healthy newborn infants (17, 21). In 3 of the infants GFR was below 25 ml/min/1.73 m², in one only 5 ml/min/1.73 m². After dilution the GFR was 41.6±14.6 ml/min/1.73 m². The most pronounced effect of isovolemic hemodilution was upon diuresis. The mean value of diuresis expressed in ml/hr/1.73 m² was 104±54 ml before dilution which should be compared with the normal value for healthy newborn infants during high fluid intake which is 168 ±53 ml (4). After dilution diuresis increased to 160±35 ml ($p<0.01$). Fig 3 also demonstrates the increase in urinary sodium excretion. The mean value before dilution was 0.85±0.85 and after 1.52±1.54 mEq/hr/1.73 m². Sodium excretion after dilution is the same as found in healthy newborn infants (3). The rise in sodium excretion was not statistically significant ($p<0.10$).

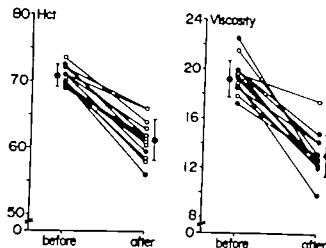


Fig 1. Individual values of hematocrit and viscosity before and after isovolemic hemodilution in 14 newborn infants (O). ●=mean values. Viscosity determinations have been performed at shear rate 23 sec⁻¹.

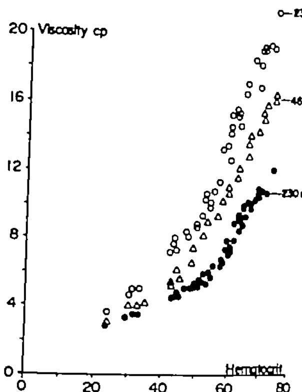


Fig 2. The relationship between hematocrit and viscosity at shear rates 23 sec⁻¹, 46 sec⁻¹ and 230⁻¹ in the newborn infants.

In Table 1 urinary sodium excretion in relation to GFR (UNaV)/100 ml GFR is given. Only in one baby was there an increase following dilution. In that infant however dilution had caused a drop of the hematocrit to a subnormal value. Diuresis (V) has also been related to GFR. It is evident that isovolemic hemodilution augmented diuresis out of proportion to GFR.

Serum albumin, serum sodium and blood osmolality were not changed by isovolemic hemodilution. Nor was there any effect on arterial blood pressure.

DISCUSSION

In newborn infants the normal range for hematocrit is 55–65% (12). Thus when compared with adults even normal newborn infants are physiologically polycythemic. Polycythemia in infants is regarded as pathological when the hematocrit is 70% or higher (14, 22).

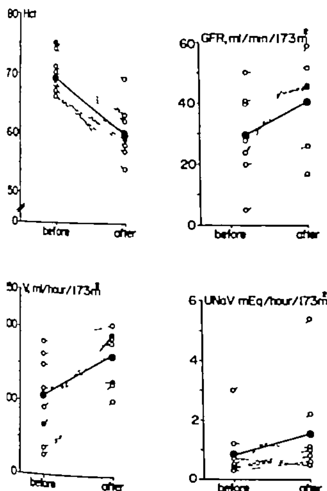


Fig. 3. The effect of hemodilution on the following parameters, hematocrit, glomerular filtration rate, urinary sodium excretion, diuresis. O = individual values. ● = mean values.

The exponential relationship between hematocrit and viscosity is well established. When there is an increase of the hematocrit from 20 to 40% the effect on the viscosity at all shear rates is very small, whereas there is a rapid increase of viscosity at each unit rise of the hematocrit when the hematocrit exceeds 50% (24). This fact was confirmed for newborns in this laboratory with shear rates ranging from 23 to 230 sec⁻¹ (6). The shear rates at which viscosity was studied may at least correspond to the blood flow conditions in the renal artery (23). It is possible that even lower shear rates are true for the blood flow in the pre- and postglomerular arterioles. However, it has been demonstrated that, if anything, the low shear rates will accentuate the effect of hematocrit on viscosity (20). Thus, it is apparent that changes in hematocrit between 60 and 70% may have important consequences for viscosity of the flow and thus for the resistance to flow.

Renal vasculature is unique in that it consists of two series-coupled arterioles, the pre- and postglomerular arterioles, with the glomerular capillary network between them. In the glomerular capillaries the blood becomes concentrated by the filtering process. Thus, the hematocrit of the blood in the postglomerular arterioles will always be higher than in arterioles in other parts of the

a decrease in blood viscosity of about 30% The relationship between hematocrit and viscosity at different shear rates is shown in Fig 2

Fig. 3 depicts the changes in renal function caused by hemodilution When the hematocrit was reduced there was an almost significant increase in GFR ($p<0.05$) The mean value before dilution had been performed was 29 7±13 3 ml/min/1 73 m² which is at the lower normal range in healthy newborn infants (17 21) In 3 of the infants GFR was below 25 ml/min/1 73 m² in one only 5 ml/min/1 73 m² After dilution the GFR was 41 6±14 6 ml/min/1 73 m² The most pronounced effect of isovolemic hemodilution was upon diuresis The mean value of diuresis expressed in ml/hr/1 73 m² was 104±54 ml before dilution which should be compared with the normal value for healthy newborn infants during high fluid intake which is 168 ±53 ml (4) After dilution diuresis increased to 160±35 ml ($p<0.01$) Fig 3 also demonstrates the increase in urinary sodium excretion The mean value before dilution was 0 85±0 85 and after 1 52±1 54 mEq/hr/1 73 m² Sodium excretion after dilution is the same as found in healthy newborn infants (3) The rise in sodium excretion was not statistically significant ($p<0.10$)

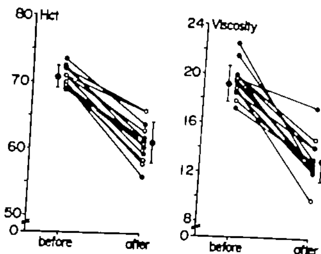


Fig 1 Individual values of hematocrit and viscosity before and after isovolemic hemodilution in 14 newborn infants (O) ●=mean values. Viscosity determinations have been performed at shear rate 23 sec

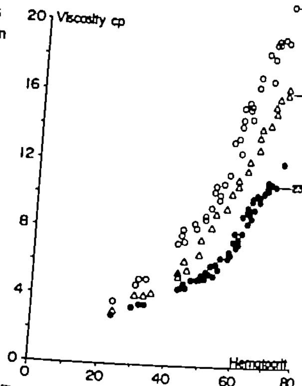


Fig 2 The relationship between hematocrit and viscosity at shear rates 23 sec 46 sec⁻¹ and 230⁻¹ in the newborn infants

In Table 1 urinary sodium excretion in relation to GFR (UNaV)/100 ml GFR is given Only in one baby was there an increase following dilution In that infant however dilution had caused a drop of the hematocrit to a subnormal value Diuresis (V) has also been related to GFR It is evident that isovolemic hemodilution augmented diuresis out of proportion to GFR

Serum albumin serum sodium and blood osmolality were not changed by isovolemic hemodilution Nor was there any effect on arterial blood pressure

DISCUSSION

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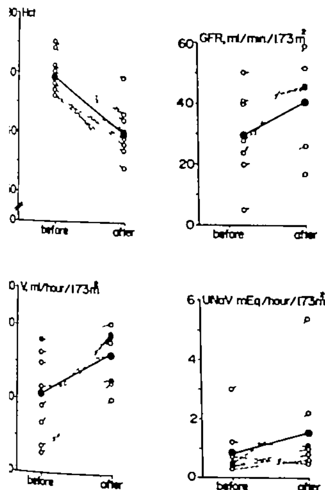


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Table 1 *Urinary sodium excretion and diuresis in relation to glomerular filtration r (GFR) in 5 infants before and after hemodilution*

	Hematocrit %		UNaV μEq/100 ml GFR		V ml/100 ml GFR	
	Before	After	Before	After	Before	After
1	75	69	0.39	0.16	0.054	0.067
2	74	60	0.50	0.4	0.073	0.075
3	75	63	0 [illegible]1	0.18	0.018	0.035
4	68	54	0.13	0.70	0.070	0.065
5*	66	57	1.24	0.80	0.119	0.101

*The infant whose hematocrit value fell spontaneously and who had the lowest GFR (5 ml/min/1 73 m²)

body As a consequence changes in systemic blood hematocrit in such a range that the effect on viscosity will be marked most probably will exhibit a more pronounced influence upon the resistance to flow in the postglomerular arterioles than in arterioles in other parts of the body Studies on renal function in adults with secondary polycythemia due to oxygen desaturation from high altitude living (5) or cardiac malformations with right to left shunts (1) have uniformly demonstrated an elevated filtration fraction which in its turn probably is due to alterations in the renal blood flow GFR has been found to be slightly depressed or almost normal Renal plasma flow has always been found to be reduced (1 5)

In the newborn period the kidney is to be regarded as immature (9) GFR is low (17 21) The mean value for full-term infants during the first week of life has previously in this laboratory been found to be 31±9 3 ml/1 73 m² body surface In adults a GFR of 25 to 30 ml/1 73 m² body surface indicates incipient renal failure In 3 of 7 of the polycythemic infants GFR before hemodilution was below 25 ml/1 73 m²/body surface In one of them it was as low as 5 ml/1 73 m² body surface GFR almost uniformly increased following hemodilution The difference of mean value was 12 ml/1 73 m² body surface

It is well documented that the glomerular plasma flow is the main determinator of GFR (8) which also appears to be true in the very young animal (2) Thus the increase in GFR following isovolemic hemodilution most likely is the result of an increase of glomerular plasma flow which in turn will be due to changes in resistance to flow as well as to the increase of plasma per unit blood

Diuresis as well as urinary sodium excretion was elevated after hemodilution. This effect may be due to several factors out of which the improvement of GFR most likely is the most important one In animal experiments sodium excretion has been found to increase when the hematocrit is reduced from approximately 40% to about 30% although GFR remains unchanged (8) In newborn full-term infants an inverse correlation between sodium excretion and hematocrit has been demonstrated (3) It was then speculated that the high hematocrit might alter the physical forces that determine proximal tubular sodium reabsorption (15) in such a way that the reabsorption will be enhanced The findings in the present study do not however permit any further speculations on the mechanism by which a high hematocrit may contribute to sodium retention in newborn infants Hemodilution was followed by a more pronounced increase of water excretion than of urinary sodium Since water excretion increased out of proportion to the increase of GFR the elevation of diuresis must be attributed to changes in tubular reabsorption Variations in intrarenal physi

cal forces as well as an increase of medullary blood flow due to the reduced viscosity may be the main cause of these changes.

A number of clinical implications may be discussed in relation to a high hematocrit in newborn infants. With late clamping of the cord a number of babies will have marked secondary polycythemia including a very high hematocrit and large blood volume. On inspection they will be recognized by their "plethoric" face. Severe cases may develop acute heart failure and oedema. The drugs which are used in cardiac failure, i.e. digitalis and various diuretics, will all influence renal function (25). Since polycythemic newborn infants can be considered to be at risk of developing renal failure, the action of these drugs is unpredictable. Therefore, hemodilution may be recommended as the treatment of choice in polycythemic infants with manifest cardiac failure. In cases with incipient failure, oral fluid administration may prevent the development of a manifest failure. The rate of administration should be rather slow since polycythemic newborns are at risk of developing an acute overexpansion of the circulatory space due to their impairment of renal function. The low renal excretion capacity should also be considered when various drugs other than digitalis and diuretics have to be prescribed to newborn polycythemic infants.

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CASE REPORT

HEREDITARY SYNDROME CONSISTING IN RECURRENT ATTACKS RESEMBLING BRACHIAL PLEXUS NEURITIS SPECIAL FACIAL FEATURES AND CLEFT PALATE

ANDERS ERIKSON

From the Department of Paediatrics Centrallasarettet Boden S eden

ABSTRACT Erikson Anders (Department of Paediatrics, Centrallasarettet, Boden, Sweden). Hereditary syndrome consisting in recurrent attacks resembling brachial plexus neuritis, special facial features, and cleft palate Acta Paediatr Scand, 63:885 1974.—A father and his two daughters with recurrent reversible attacks resembling brachial plexus neuritis are presented. In this family cleft palate and a previously described characteristic facial appearance accompanied the disorder No other abnormalities nor any specific cause of the attacks were found. EMG of shoulder arm and hand muscles revealed signs of partial denervation Physiotherapy seemed to accelerate the spontaneous recovery The condition has a better prognosis than some similar disorders for which it is easily mistaken.

KEY WORDS: Brachial plexus neuritis, shoulder neuritis, hereditary neuritis, muscle weakness of upper extremities

The occurrence of a familial form of brachial plexus neuritis was reported as early as in 1887 by Dreschfeld (2) and in 1893 by Ross & Bury (7)

In 1960 Taylor (9) described a family with 119 members in 5 generations Twenty-six family members had one or several attacks, as a rule localized to the brachial plexus region on one side or both sides The symptoms during attacks were severe pain muscular weakness sensory loss and muscular atrophy which all usually disappeared after some months Hoarseness due to involvement of the recurrent laryngeal nerve difficulty in swallowing and breathing as well as symptoms from the autonomic nervous system also occurred in a few patients Pregnancy the puerperium and acute infectious diseases are mentioned as provoking factors No significant abnormalities of the blood urine or cerebrospinal fluid were found Taylor considers the inheritance as being due to an autosomal dominant gene with high penetrance.

Jacob et al (5) reported seven cases in two unrelated families All their patients had a characteristic facial appearance with deeply set eyes lying closely together Other similar cases have been reported (3 4 6 8 10)

CASE REPORTS

Case I

(Figs. 1 and 2). An 11-year-old girl 3rd of 5 siblings, was born with a cleft palate At age 4 years she was found to have a right-sided optic atrophy of which no cause was apparent.

In June 1972 she had a sudden attack of pain in her right shoulder radiating to the arm and hand On the preceding day she had been playing ball The pain gradually disappeared, but she developed paresthesia and increasing muscular weakness in the arm and hand

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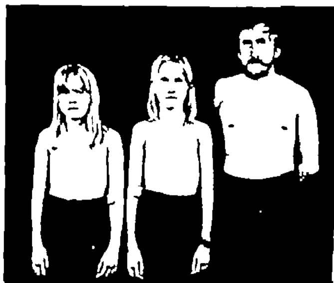


Fig 1 The three patients. From the left, cases I II and III

On admission 4 weeks after the onset of symptoms it was noted that she had a peculiar facial appearance with deeply set eyes with a slight mongoloid slant. Neurological examination revealed weakness and muscular atrophy of the right arm wristdrop (Fig. 2) a 30° limitation of the extension of the right elbow and a winged scapula. No muscle reflexes could be elicited on the right arm. There was sensory loss over the areas of the hand and forearm mainly innervated by the nerve roots C 6 and C 7. The left arm was completely normal as were the rest of the findings at neurological examination. The following laboratory studies gave normal results. Aminoleuvulinic acid porphobilinogen, and porphyrins in the urine activity in the serum of transaminase (GOT and GPT) creatine kinase and aldolase serum triglycerides cholesterol lipoprotein and protein electrophoresis. Immunoglobulin determination revealed a slight excess of IgE but otherwise normal titres. The findings in the cerebrospinal fluid were mononuclear cells per mm³ total protein 23 mg/100 ml and a normal electrophoretic pattern.

Radiography of the right shoulder neck and thoracic spine revealed reduced mineralization of the inactivity type in the shoulder but otherwise normal conditions. On chromosome analysis a normal female chromosome pattern was found. EEG was normal. EMG revealed partial denervation of several muscles of the right arm and hand the distribution of the abnormality was compatible with a lesion of the brachial plexus. The conduction velocity of peripheral motor nerves in the arms and legs was normal. Otological examination



Fig 2 Case I with a wristdrop during her attack.



Fig 3 Case II The winged scapulae are clearly seen

vealed normal hearing and no paresis of the recurrent laryngeal nerve.

Physiotherapy was the only treatment given. One year later she appeared recovered.

Case II (Figs. 1 and 3)

This 12-year-old girl was the older sister of the previously described patient. She was born with a cleft palate. When she was 3 years old she knocked her right arm on falling. The next day intense pain suddenly started and weakness gradually developed in the same arm. On examination 3 weeks later she had severe muscular weakness and atrophy in the right arm. Her condition was diagnosed as muscular dystrophy of the humero-scapular type. The muscle strength gradually returned. Several similar attacks have since occurred both in her left and in her right arm.

She was examined by the author when her sister's symptoms started. Her facial appearance was similar to that of her sister. She had bilateral muscular atrophy in the shoulder region (Fig. 3), reduced strenght in the hands and arms, a limitation of the extension of the left elbow and sensory loss on the ulnar half of the dorsal aspect of the right forearm. Otherwise her physical status was normal. Laboratory findings in the blood and urine were normal as in case I. EEG was normal. EMG showed partial denervation of several muscles of both upper extremities. The abnormality was more pronounced in proximal muscles than in distal ones. Its distribution was compatible with bilateral damage to the brachial plexus. The conduction velocity of her right median nerve was borderline low (46 m/sec), whereas it was normal in other nerves of her arms and legs. With physiotherapy the girl has since improved.

Case III (Fig. 1)

This was a 37-year-old man, father of the 2 sisters presented. He was born with a cleft palate. At the age of 3 years he had a sudden attack of pain and weakness in his right arm. When he was 15 years old he had an attack first on the right and then on the left side. He was then admitted to a hospital and diagnosed as a case of progressive muscular dystrophy. Since then he has had some further mild attacks. When his younger daughter had her first attack, he was examined by the author. He had the same facial appearance as his daughters (Cases I and II). He had slight muscular atrophy in both shoulders. EMG of muscles innervated by the brachial plexus showed bilateral signs of previous partial denervation.

DISCUSSION

Familial occurrence of recurrent brachial plexus neuritis has not been reported previously in Scandinavia. Several families have been described in USA and Great Britain. The affected members of the family reported here have a characteristic facial appearance with deeply and narrowly set eyes which have a slight mongoloid slant. Their appearance resembles that of patients with hereditary brachial plexus neuritis described earlier (5, 6). All three affected members of this family had cleft palate. None of the relatives were known to have the characteristic facial appearance or cleft palate. To the author's knowledge the occurrence of cleft palate has not previously been reported together with recurrent brachial plexus neuritis.

The cause of the attacks is unknown. Some form of hyperergic reaction has been discussed. In the three cases presented the attacks were usually preceded by mild trauma. No other possibly provoking factors were present. The location and nature of the underlying mechanism remain obscure.

Steroids may relieve the initial pain but have no effect on the weakness (6). Physiotherapy is important both initially to prevent contractures and subsequently to improve the muscle strength. The long term prognosis is usually good and the weakness abates completely or partly.

The occurrence of this rather benign and rare disorder also in Scandinavia is emphasized. Patients with the condition may exist misdiagnosed as were the present cases II and III and thus be given a too serious prognosis.

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CASE REPORT

ASYMMETRIC TESTICULAR DYSGENESIS

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From the Paediatric Institute and Institute of Anatomy and Histology Medical Academy in Cracow Poland

ABSTRACT Rybak, M., Sokołowski, J., Szmigiel C., Kleczkowska, A., Nowicki, J and Pilch, J (Paediatric Institute and Institute of Anatomy and Histology Medical Academy Cracow Poland). Asymmetric testicular dysgenesis. Acta Paediatr Scand 63: 889 1974.—An 8-months-old child with abnormal sexual development and male karyotype is described. On the basis of clinical, histological and cytogenetic investigations a diagnosis of asymmetric testicular dysgenesis has been established. The abnormalities in gonadal development are probably the result of inadequate and delayed action of inducing substance in early embryonic life. In accordance with the appearance of the external genitalia and the karyotype male sex has been accepted. It seems reasonable to remove the female rudiments because of a high percentage of malignancy in these cases. Therapy with androgens will be necessary at the time of puberty

KEY WORDS: Gonadal dysgenesis, intersexuality

The classical picture of asymmetric gonadal dysgenesis (AGD) i e the mixed or atypical gonadal dysgenesis (16) is characterised by a unilateral streak gonad and an intraabdominal or sometimes inguinally located testis on the other side Federmann observed this picture in 5% of 42 cases diagnosed as AGD (2) Other possible combinations might be taken into consideration under this clinical heading: unilateral gonadal agenesis hypoplastic abdominal gonads with rudimentary testicular tissue in one of them a streak gonad on one side and a gonadal tumor on the other (13 15).

In all the variants mentioned above the uterus the upper part of the vagina and at least one Fallopian tube are preserved In most cases clitoromegaly urogenital sinus and labioscrotum can also be detected The phenotype is usually hermaphroditic yet female features are more prominent. A male phenotype with hypogonadism is less often seen (2)

The most common cytogenetic lesion in AGD is mosaicism usually including XO cells and a clone with the Y chromosome A karyotype XY is relatively rare Fergusson-Smith et al (3) described 4 cases Federmann (2) found the XY karyotype in 5 patients

CASE HISTORY

The patient we present has an AGD connected with unusual genital abnormalities and the XY karyotype.

The child, KB 020567 was admitted initially to the Paediatric Institute in Cracow at the age of 8 months because of abnormal genital development. A hypoplastic phallus without any cavernous bodies was 1.5 cm long. The urethral orifice was on the tip of the glans penis. The rudimentary scrotum resembled fused labia majora. Testes were palpable neither in the scrotum-like folds, nor in the inguinal canals (Fig. 1). The child's sex was determined at birth as male The patient was born as the fifth child of a normal pregnancy Both parents were healthy and not related. At delivery the mother was 39



Fig 1 The patient K B with asymmetric testicular dysgenesis, aged 8 months.



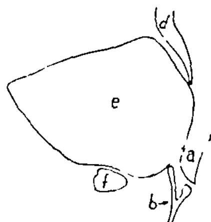


Fig 2 Genitography: the vagina (*a*) with the outlet (*b*) to the posterior upper part of the urethra (*c*) (*d*) Reflu vesico-ureteric (*e*) urinary bladder (*f*) symphysi pubis

the father 38 years old No congenital malformations had been recorded in the family The siblings development proved to be normal The patient was mentally and physically normal A daily excretion of 17-CS and 17 OH steroids in urine at the age of 8 months and in the twentyfirst month as well as serum and urine ionograms was normal *Sex chromatin examined from buccal smears* revealed no Barr bodies 151 metaphase plates from three consecutive lymphocyte cultures (6 12) were analysed and the 46 XY karyotype was determined. Contrast radiography of the genito-urinary tracts revealed a vagina with the outlet to the posterior upper part of the urethra (Fig. 2) A uterus measuring 3 by 2 by 1.5 cm of normal shape and on the right side the oviduct with a hypoplastic gonad measuring 0.5 by 0.5 cm in the position of an ovary were detected during explorative laparotomy Neither a gonad and genital tract, nor a broad *ligament were found on the opposite side* In the left corner of the uterus a ballshaped structure 0.5 cm in diameter could be seen

On the request of the parents our patient was discharged from hospital and 2 years later was admitted again At that time his physical and mental development was found to be normal for the age. The external genitalia looked like those described in the first year of *life According to the opinion of majority of authors re*garding the frequency of malignancy in dysgenetic gonads the right gonad was removed Histological material obtained under surgery comprised sections from the right gonad (Figs 3 and 4), and sections of the ball-shaped structure from the left corner of the uterus. These last cross sections show a male deferent ductus of the appearance similar to the microscopic picture on the right side but smaller in size.

Dermatoglyphic patterns on the left and right hands (after Penrose & Loesch (8)) were as follows. patient—*WAUUW III t 4 (5) WUUUU III t 4 (5) patient s* father—URUWU I I' II III IV H e e f t t' 6 (5) WAUUU I II III H e t t 5 (5) patient s mother—WRWUU III t 4 (5) WUUUU IV t 4 (4). Values of the TRC and sums of left and right *a–b* ridge counts and maximal *atd* angles amounted respectively in patient 112 98 and 90° (corrected for age and male sex according to Penrose (9)), in patient s father 77 85 and 127° and in patient s mother 131 78 and 81

DISCUSSION

Jacobs & Ross (4) suggested that foetal masculinisation is determined by the genes



Fig 3 Well developed seminiferous tubules of the right gonad in cross and oblique section are lined with cylindrical epithelium rarely containing large light cells (presumably spermatogonium). This system of seminiferous tubules and the structure of the connective tissue sheath are typical for the male gonad (low power).

situated on the short arm of the Y chromosome The normal male gonadal development is only possible when the XY configuration is present. The most prominent activity of the foetal testis is to promote the normal development of the gonadal ducts during the first 12 weeks of foetal life (5 14 8) The masculinisation depending on foetal testis is merely local and strictly unilateral

There is no clear explanation why some persons with the XY configuration exhibit female sexual development As Overzier emphasized in mosaic patients one clone of cells is likely to disappear in an early period of life In the persons with AGD and XY karyotype local mosaicism in the gonads may also occur Finally the morphologically normal sex chromosomes may be defective in their activity (5) According to Federmann an asymmetric development of internal sexual organs may result either from an insufficient activity of the inducing substance or its delayed synthesis Thus these factors are not effective unless acting at a specific time There is also the possibility of action by a local teratogenic factor which may induce the atrophy of testicular tissue in the early stage of gonadogenesis

In our case the gonadogenesis had obviously been disturbed at an early stage in embryonic life No testis could be found on the left side The right hypoplastic gonad shows histologically both male and female elements an epididymal canal vas deferens and an oviduct. We presume therefore that the function of the dysgenetic foetal gonad was not only insufficient but also delayed in time the suppression of the Mullerian structures did not follow at the proper time The defective development of the external genitalia is due to the inadequacy of the androgenic effect of the foetal testis It seems



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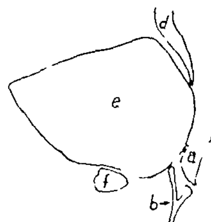


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DISCUSSION

Jacobs & Ross (4) suggested that foetal masculinisation is determined by the genes

androgen steroids at puberty. It seems also reasonable to remove the uterus and the Fallopian tube according to Federmann's suggestion. The author cites two reports about malignancy occurring in these organs in analogical cases.

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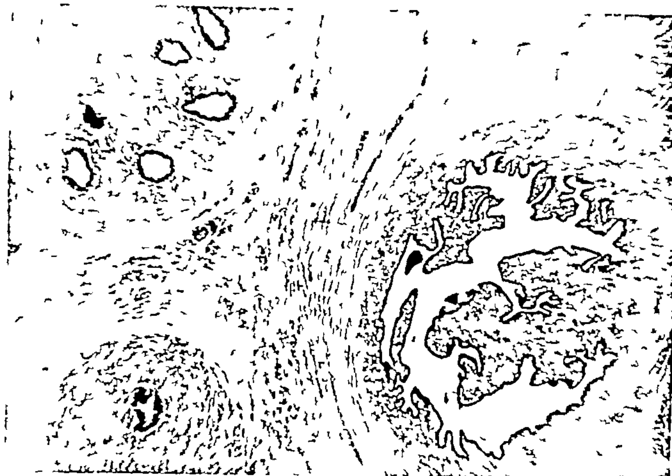


Fig 4 The section shows further part of the right gonad with male genital ducts (ductus epididymidis or even the deferent duct) The same section contains the oviduct with the structure of folds typical for the labyrinth (right side) (low power)

rather difficult to explain why the absence of the left gonad as well as of the gonadal ducts occurred in our case The only maintained male structure is a rudimentary spermoduct at the left corner of the uterus These findings suggest that the foetal testis had suppressed the Mullerian ducts at an early stage of embryonal development and then disappeared

In 1962 Wilkins (14) described bilateral testicular dysgenesis with karyotype 46 XY maintained uterus and two Fallopian tubes This syndrome was named mixed gonadal dysgenesis Federmann (2) called this malformation dysgenetic male pseudohermaphroditism The genetic and anatomical features in both the syndromes are alike and therefore the precise differentiation is difficult Sohval (11) and Bergada et al (1) classified the cases of unilateral gonadal agenesis connected with dysgenetic testis on the other side as mixed gonadal dysgenesis Usually cases with male phenotype and a pure XY chromosome constitution present as testicular dysgenesis whereas those with mosaicism and external sexual ambiguity rather presents as AGD In the first case the histological picture shows no female structures in the gonads in the second one there are both ovarian and testicular rudiments These abnormalities are the result of asymmetry and disorder in the gonadal development in the first weeks of embryonic life

In the light of this hypothesis our case could be diagnosed as asymmetric testicular dysgenesis Because of the appearance of male external genitalia the diagnosis of the male sex has been maintained The complete lack of gonads calls for substitutional therapy with

fficulty could be overcome either by giving a arger dose of a non-water miscible preparation or by using intramuscular adminstration

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LETTER TO THE EDITOR

Tocopherol and the Anaemia of Prematurity

A relationship between tocopherol deficiency and the anaemia of prematurity was first demonstrated by Oski & Barness (4) Since then several authors have shown that the prophylactic administration of tocopherol can at least in some cases reduce the severity of the anaemia (1 2 3) Little work has been done however on the effect of treatment of the anaemia with tocopherol in relatively large doses over a short period Since tocopherol deficiency does not manifest itself until the age of 3–4 weeks there would seem little point in giving tocopherol to premature infants before this age if the same effect can be achieved by giving it when the anaemia is beginning to develop The present study was undertaken to test this hypothesis by means of a double blind controlled trial

Infants were selected for the trial if they satisfied the following two criteria (*a*) birth weight less than 2.5 kg and (*b*) they would be in hospital at least until the age of 5 weeks Consecutively numbered bottles were allocated to the infants some of these bottles contained tocopherol and the remainder contained a dummy suspension The code was contained in a sealed envelope which was not opened until the trial was completed The dosage of tocopherol which was chosen was 100 mg/day for one week starting at the age of 4 weeks and this was in the form of α-tocopherol acetate in a water miscible suspension All the infants in the trial also received iron and folic acid supplements from the age of 3 weeks

At the start of the trial the mean plasma tocopherol was 0 34 mg/100 ml in the control group (19 patients) and 0 42 mg/100 ml in the treated group (18 patients) This difference was not significant At the end of the week s treatment the values were 0 48 mg/100 ml and 2 7 mg/100 ml in the two groups respectively The difference between these two latter values was highly significant ($p<0\ 001$) The Hb concentrations are shown in the accompanying Table

The Hb concentrations in the two groups were compared using analysis of variance and the F test the trend of the Hb concentrations in the treated group was found to be significantly different from that of the control group ($p<0\ 05$) No significant difference was found between the reticulocyte counts or platelet counts in the two groups No ill effects attributable to the administration of the tocopherol were encountered

Since the administration of tocopherol in high dosage over a short period is capable of producing a beneficial effect on the early anaemia of prematurity and is likely to be more convenient than continuous administration of smaller doses from an early age it is suggested that this method of treatment should become part of the routine management of low birth weight infants Unfortunately at present a water miscible preparation of tocopherol is not routinely available in this country but this

Table *Mean Hb concentrations of the two groups*

Age (weeks)	4	5	6	7	8	9	10	12
Hb (treated group)	10.35	9 13	9 08	9 49	10 36	9 74	11 18	10.54
Hb (control group)	10 45	9 04	8 63	8.84	8 44	9 17	9.33	10.54

General Index

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ANNOUNCEMENTS

The annual meetings of the American Pediatric Society and the Society for Pediatric Research will be held at the Hilton Hotel in Denver April 16 through 19 1975 The schedule for these meetings is as follows.

Wednesday April 16 P M American Pediatric Society Society for Pediatric Research and American Academy of Pediatrics (Symposium on recent research advances)
Thursday April 17 A M American Pediatric Society (Symposium)
Thursday April 17 P M American Pediatric Society (Plenary session)
Friday April 18 A M American Pediatric Society and Society for Pediatric Research (Subspecialty sessions)
Friday April 18 P M Society for Pediatric Research (Plenary session)
Saturday April 19 A M & P M American Pediatric Society and Society for Pediatric Research (Subspecialty sessions)

The deadline for receipt of abstracts by the secretaries of the societies is January 6 1975 The combined registration fee (APS & SPR) is $15 For additional information write to Charles D Cook M D (Secretary American Pediatric Society 333 Cedar Street New Haven Connecticut 06510) or Jo Anne Brasel M D (Secretary Society for Pediatric Research Columbia University College of Physicians & Surgeons, 630 West 168th Street New York, New York 1003?)

Fellowship in Developmental Psychology for Pediatricians This two year post doctoral fellowship is designed to train academically oriented pediatricians in the knowledge and research skills of developmental psychology Formal graduate level course work will be combined with a supervised research project planned and conducted by the fellow

Physicians who have completed two years of pediatric training and who are interested in a career in academic medicine are eligible to apply One fellow will be accepted each year beginning July 1975 This program is supported by the Commonwealth Fund

For further details write Robert W Chamberlin M.D University of Rochester Medical Center Department of Pediatrics 260 Crittenden Boulevard Rochester New York 14642 USA

The 6th Annual Meeting of European Working Group for Cystic Fibrosis will be held in Dublin on June 30th and July 1st 1975 Further information can be obtained from The Irish Medical Association Conference Centre 10 Fitzwilliam Place Dublin ?

A prize of Sfr 15 000 will be awarded again in September 1975 by the Central Union of Swiss Milk Producers, Berne, to a scientist from one of the following countries members of the International Dairy Federation Argentina, Australia, Austria Belgium Brazil Bulgaria, Canada, Czechoslovakia, Denmark, Finland France India, Ireland Israel Italy Japan Kenya, Luxemburg, Netherlands New Zealand Norway Poland South Africa, Spain Sweden Switzerland United Kingdoms, U.S.S.R., West-Germany

The subject chosen for the 1975 prize is *Digestion of Milk* All scientists (chemists physicians biologists) who have worked in this field are eligible Applications should be sent to the President of the jury Professor M. Demole Unité de Diététique Hôpital Cantonal CH 1211 Genève 4 until January 31 1975 with *3 copies of (a)* curriculum vitae *(b)* list of works, *(c)* reprints of 2 or 3 papers on the theme of the prize published in the last 5 years (no typewritten papers) These documents should be written in *English, French or German* or should be accompanied by a translation into one of these 3 languages. (They will not be returned to the authors)